THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as

regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XI. The accounts of the Treasurer shall be audited annually by a certified public accountant.

XII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1943.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and inspection of it by members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B and C.

The following are some general statements and observations based on the detailed reports:

I. Assets

1. Endowment Assets

As of December 31, 1943, the total of all the Endowment Assets was $1,003,708.63, a loss of $68,282.27 from the total for the preceding year. This substantial loss was due almost entirely to the sales of three New York City real estate holdings on which the Laboratory Trust Funds with the Central Hanover Bank and Trust Company had first mortgage participations. The three properties sold were a 15-story office building at 89 Madison Avenue, a 7-story loft building at 25 Spruce

Street, and an apartment house at 127 West 79th Street. The Laboratory's total investment in the three mortgages was $98,315.24; from the foreclosures and sales the Laboratory received $16,827.70 cash and new mortgages for $16,251.81; the total capital loss was thus $65,235.73.

During 1943 the *market* values of the Laboratory's securities, except for the mortgage and real estate participations, made a substantial gain. As of December 31, the totals of government and corporate bonds, common and preferred stock holdings were $754,904.23 book or cost value, with a market value of $750,286.38.

The total of mortgage and real estate participations was $219,719.95 book value, approximately 22 per cent of the total. There are no market quotations for these securities, but in view of the loss of over 60 per cent of the cost of the three real estate holdings sold last year, the generally deteriorating position of New York City property, and the difficulties now besetting some of the other 16 mortgages in which the Laboratory has participations (one of them has already been foreclosed) it would be unwise to estimate their actual value at much more than 50 per cent of cost, or about $110,000. The actual value of the Endowment Funds including uninvested cash of $29,084.45, would thus be approximately $889,370.83.

2. Plant Assets

The total of Plant Assets (excluding the Gansett and Devil's Lane Tracts) was $1,341,425.88 after deduction of $633,901.42 accumulated Depreciation Reserve, a decrease of $16,336.11. During the year, depreciation charges deducted amounted to $26,969.11. The Reserve Fund was increased by $8,998.71 cash transferred from current income, representing profits on sale of Gansett lots, and a portion of the dividends received on the General Biological Supply House and Crane Company stocks.

3. Current Assets

Current Assets including cash, inventories and investments not in the Endowment Funds, amounted to $194,111.69, an increase of $29,442.67. Current Liabilities were only $3,251.25. After the setting up of a special reserve of $2,358.34 for repairs and replacements on the properties leased by the Navy, the total of Current Surplus was $188,502.10, an increase of $28,829.33 over 1942.

II. Income and Expenditures.

Total Income was $159,296.94, an increase of $13,227.18 over the preceding year. Total Expenses, including the $26,969.11 added to Depreciation Reserves, were only $139,973.27 compared with $159,296.94, so that for 1943 there was an actual net operating surplus of $19,323.67 in gratifying contrast with the deficit of $17,211.93 for 1942.

This excess of income over expenditures is due to a number of factors. One was the $20,150.00 received or accrued as rentals from the United States Navy. Not all of this total was a clear increase, of course, as normal income from the leased properties was eliminated, but at least a portion of it represented increased income which will not again be available. Income from the General and Library Endowment Funds reversed the recent downward trend and rose slightly to $36,610.98. More substantial gains over 1942 were shown in the income from the Supply Department which gave a profit of $14,436.21 compared with the 1942 deficit of $1,699.05 on only a slightly increased volume of sales, and in the dividends

from the General Biological Supply House stock which were $18,796.00 as against $10,922.00.

Even more important than the increases in income were the careful reductions in expenditures made by the Laboratory Administration. Maintenance expenses were still further cut in nearly every department, and purchases and improvements were kept at a minimum to an extent that would be impossible under normal operating conditions.

Both income and expense totals must therefore be regarded as abnormal because of the special circumstances prevailing in the year 1943.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1943

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 993,773.34	
Securities and Cash in Minor Funds		9,935.29	
			$1,003,708.63
Plant Assets:			
Land	$ 111,425.38		
Buildings	1,322,348.21		
Equipment	185,890.98		
Library	321,626.42		
	$1,941,290.99		
Less Reserve for Depreciation	633,901.42	$1,307,389.57	
Cash in Reserve Fund		13,272.22	
Cash in Book Fund		20,764.09	
			$1,341,425.88
Current Assets:			
Cash		$ 23,312.61	
Accounts Receivable		12,217.19	
Inventories:			
Supply Department	$ 42,826.62		
Biological Bulletin	17,971.16		
		$ 60,797.78	
Investments:			
Devil's Lane Property	$ 45,967.76		
Gansett Property	2,694.70		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	17,770.00		
Retirement Fund	13,242.17		
		$ 92,374.63	
Prepaid Insurance		4,928.83	
Items in Suspense		480.65	
			$ 194,111.69
Total Assets			$2,539,246.20

Liabilities			
Endowment Funds:			
Endowment Funds	$ 991,628.91		
Reserve for Amortization of Bond Premiums ..	2,144.43		
		$ 993,773.34	
Minor Funds		9,935.29	
			$1,003,708.63
Plant Liabilities and Surplus:			
Donations and Gifts		$1,172,564.04	
Other Investments in Plant from Gifts and Current Funds ..		168,861.84	
			$1,341,425.88
Current Liabilities and Surplus:			
Accounts Payable		$ 3,251.25	
Reserve for Repairs and Replacements		2,358.34	
Current Surplus (Exhibit C)		188,502.10	194,111.69
Total Liabilities			$2,539,246.20

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1943

	Total		Net	
Income:	Expense	Income	Expense	Income
General Endowment Fund		$ 29,310.84		$ 29,310.84
Library Fund		7,300.14		7,300.14
Instruction	$ 6,598.09	5,085.00	$ 1,513.09	
Research	4,488.30	8,662.50		4,174.20
Evening Lectures	25.00		25.00	
Biological Bulletin and Membership Dues	5,292.02	6,859.33		1,567.31
Supply Department	27,215.89	41,652.10		14,436.21
Mess	17,237.17	19,093.67		1,856.50
Dormitories	22,605.46	15,439.65	7,165.81	
(Interest and Depreciation charged to above 3 Department)	24,866.25			24,866.25
Dividends, General Biological Supply House, Inc.		18,796.00		18,796.00
Dividends, Crane Company		500.00		500.00
Rents:				
Bar Neck Property	717.77	3,600.00		2,882.23
Janitor House	29.17	360.00		330.83
Danchakoff Cottages	267.47	300.00		32.53
Lecture Hall and Botany building		999.96		999.96
Rooms in Laboratory, Special		245.00		245.00
Sale of Library Duplicates and Micro Flms		446.14		446.14
Microscope and Apparatus Rental		613.55		613.55
Sundry Income		33.06		33.06
Maintenance of Plant:				
Buildings and Grounds	19,529.79		19,529.79	
Apparatus Department	4,683.32		4,683.32	
Chemical Department	1,588.86		1,588.86	
Library Expense	6,463.00		6,463.00	
Workmen's Compensation Insurance	429.53		429.53	
Truck Expense	152.22		152.22	
Bay Shore Property	86.84		86.84	
Great Cedar Swamp	17.10		17.10	

General Expenses:				
Administration Expense	14,386.77		14,386.77	
Endowment Fund Trustee and Safe-keeping	1,014.45		1,014.45	
Bad Debts	720.29		720.29	
Special Repairs, Supply Dept. Stone Building	1,963.56		1,963.56	
Reserve for Repairs and Replacements, Buildings occupied by Navy	2,358.34		2,358.34	
Reserve for Depreciation	26,969.11		26,969.11	
	$139,973.27	$159,296.94	$ 89.067.08	$108,390.75
Excess of Income over Expense carried to Current Surplus	$ 19,323.67		$ 19,323.67	
	$159,296.94		$108,390.75	

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account, Year Ended December 31, 1943

Balance, January 1, 1943			$159,672.77
Add:			
Excess of Income over Expense		$19,323.67	
Gain on Gansett Lots Sold		279.41	
Bad Debt Recovered		.90	
Transfer of Biological Bulletins, held at Lancaster Press for Uncompleted Serial Exchanges, 1940, 1941, and 1942 from Plant Account, Library to Biological Bulletin Inventory		2,552.50	
Reserve for Depreciation charged to Plant Funds		26,969.11	
			49,125.59
			$208,798.36
Deduct:			
Payments from Current Funds during Year for Plant Assets:			
Buildings	$ 2,460.20		
Equipment	984.64		
Library	4,583.95		
	$ 8,028.79		
Less Received for Plant Assets Sold	140.00	$ 7,888.79	
Pensions Paid	3,460.00		
Loss on Retirement Fund Securities	181.48		
	$ 3,641.48		
Less Retirement Fund Income	232.72	3,408.76	
Transfers to Reserve Fund, Portion of Dividends of Prior Years from General Biological Supply House, Inc. and Crane Company	8,500.00		
Profit on Gansett Lots for 1941 and 1942	498.71	8,998.71	20,296.26
Balance, December 31, 1943			$188,502.10

Respectfully submitted,

Donald M. Brodie,
Treasurer

V. REPORT OF THE LIBRARIAN

The librarian's report for 1942 gave a detailed account of the readjustments that the library has gradually made to changes of the past few years in the budget, in the receipt of European and Asiatic current journals and in the available markets for purchases of "back sets." It is not necessary this year to comment on these adjustments except to state that definite effort has been made to renew exchange relationship with Russia, China and India and with the limited success that was anticipated. There should be some results to report for this in 1944–45, depending, of course, on the circumstances of the war.

The budget of $12,200 for 1943 was expended as follows: books, $314.33; serials, $1871.51; binding, $645.41; express, $64.29; supplies, $202.91; salaries, $7200.00; back sets, $815.77; sundries, $3.07; and insurance, $45.00; total, $11,162.29. The sale of duplicates brought in this year $214.03; and the income from the microfilm service amounted to $221.33, 99 orders having been filled.

From the "Carnegie Fund" $1745.92 was expended on two completed and 14 partially completed "back sets" and three books that are designated as "classics."

The Woods Hole Oceanographic Institution appropriated $1850.00 for 1943 and a balance of $70.11 remained from the 1942 budget. An expended sum of $1657.03 has been reported to the Director. A balance of $263.08 was carried on to the year 1944.

During 1943 the library received 645 current journals: 232 (10 new) by subscriptions to the Marine Biological Laboratory; 15 (none new) to the Woods Hole Oceanographic Institution; exchanges, 191 (two new) with the "Biological Bulletin" and 22 (six new) with the Woods Hole Oceanographic Institution publications; 181 as gifts to the former and four to the latter. The Marine Biological Laboratory acquired 113 books: 47 by purchase of the Marine Biological Laboratory (three "classics"); 11 by purchase of the Woods Role Oceanographic Institution; 12 gifts from the authors, 19 from the publishers, 10 from Mr. John Crane, three standard medical books from Lt. F. G. Hirsch which came from his father's library, and 11 as miscellaneous gifts. There were 20 back sets of serial publications completed: five, purchased by the Marine Biological Laboratory (two with "Carnegie Fund"); three, by the Woods Hole Oceanographic Institution; one secured by exchange with the "Biological Bulletin"; five by exchange with the Woods Hole Oceanographic publications; one as a gift from Lt. F. G. Hirsch; and five secured by duplicate material exchange and by gift. Partially completed sets were 50: purchased by the Marine Biological Laboratory, 23 (14 with "Carnegie Fund); three by the Woods Hole Oceanographic Institution; by exchange of the "Biological Bulletin," none; by exchange of the Woods Hole Oceanographic Institution publications, three; by gift and by exchange of duplicate material, 21.

The reprint additions to the library number 7927: current of 1941, 933; current of 1942, 1471; current of 1943, 381; and of previous dates, 5142. A total of 981 reprints, 296 not duplicates of our holdings, were presented to the library: 396 by Dr. H. E. Crampton; 63 by Dr. H. S. Hopkins; and 522 by Dr. Libbie H. Hyman.

At the end of the year 1943 the library contained 51,945 bound volumes and 130,650 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of The Marine Biological Laboratory:

Gentlemen:

I beg to submit herewith a report of the fifty-sixth session of the Marine Biological Laboratory for the year 1943.

1. *Research*

During the past year research and instruction have gone on as usual, but the exigencies of war teaching and war research, and the draft have cut deeply into our attendance. There were 108 investigators as compared with an average of 366 in the five years preceding the war. The number of Library readers was somewhat larger than before. In most cases, the length of stay at the Laboratory was shorter than in normal years. The Friday Evening Lectures were continued as usual, but no seminars were held, nor was there a general scientific meeting at the end of the season. Research directly connected with the war was directed by Drs. D. E. S. Brown, Clowes, Heilbrunn, and Jacobs. Other projects were sponsored by the Oceanographic Institution, the Massachusetts General Hospital, and by the Bell Telephone Laboratories. During the Spring of 1944, a number of investigators from the Oceanographic Institution, engaged in war research, have occupied some of our Laboratory rooms.

2. *Instruction*

The Laboratory has been able to continue its courses of instruction with able teaching staffs and with a reasonable number of students. Of a total of 68 students in all of the courses, 11 were men and 57 were women. In peace times the total has been about 125, somewhat less than half being men. The Invertebrate course, under Dr. Buck and an almost entirely new corps of instructors, was successfully given to a class of almost maximum size. Dr. Hamburger and Dr. Costello, with the occasional help of Drs. Barth, Metz, and Rose, carried on the Embryology course with excellent results. Pressure of war work prevented most of the instructors in Physiology from remaining in residence during the entire period of the course, but in spite of this, Dr. Parpart and his associates were able to conduct the course satisfactorily. It was a great disappointment that the course in Algae had to be omitted because of a lack of students. This is the first time since the course first began in 1889 that instruction in Botany has not been given at this Laboratory.

3. *Buildings and Grounds*

With the abatement of danger from enemy attacks in this region, the Navy closed its Base at Woods Hole and gave back to us, on January 1, 1944, our buildings which they had occupied since May 1942. During their tenancy they made a number of alterations, many of which are of permanent value. The Lecture Hall and the Botany Building were given new roofs and shingles and solid shorings to support the floors. In the Homestead new sills and other timbers, and floors, all greatly needed, were installed. The Mess was improved by a new roof and insulated walls, a completely rebuilt ice box and store room, and by the addition of two new insulated store rooms. At the Penzance Garage a new concrete floor was laid where the old one was imperfect. In addition, the water front which had been

weakened by the hurricane and only partially repaired, was thoroughly rebuilt. These improvements, which we should have had to make sooner or later, are conservatively valued at $12,000.

On the other hand, the Navy made other alterations which rendered the buildings unsuitable for our use. In the lease, the Navy agreed to restore the property to its original condition. But it was obvious that the necessary repairs and replacements could be made more satisfactorily by our own staff than by an outside contractor. Consequently, after a period of bargaining, it was agreed that the Navy should leave in place some valuable equipment, useful to the Laboratory, in lieu of actual payment for the cost of the repairs. Among the items transferred to us may be mentioned a series of gas cooking ranges equipped with a ventilating hood, and an oven. These take the place of our old coal range which was outworn. Also a water cooler, a refrigerating unit in the icebox, a hot water heating system, extensive plumbing installations in the Homestead, and 12 tons of hard coal in the Apartment House. This equipment is valued at more than $6000, an amount well above the cost of repairs and replacements made by our staff. The Lecture Hall and the Botany Building have not been completely restored since they will not be needed in 1944. The Apartment House, which was returned to use in fairly good condition, has been redecorated for the first time since it was built in 1927. The Penzance Garage is now leased to the Oceanographic Institution for the duration.

Although a number of our buildings are now in better condition than heretofore, some need further attention. Specifically, the walls of the Apartment House should be waterproofed, at least on those sides exposed to storms. Unless this is done soon much of the new decoration will be marred. The Brick Building also should be similarly treated.

4. *Financial*

The Treasurer's report shows that our finances are in some respects in excellent condition. The cash balance is larger than usual, due in part to the large dividend of the General Biological Supply House and increased sales by our own Supply Department, but especially to the rental paid by the Navy. At the same time our expenditures have been low for the same reasons that were noted last year, namely, that we have been unable to purchase new equipment and to pay for current foreign journals. Because of this favorable cash balance we have felt justified in adding a substantial amount to the Reserve Fund, and in making the extensive improvements in the Apartment House.

On the other hand, our income for 1944 will be reduced by reason of the withdrawal of the Navy and the relatively small return from fees of investigators and students. We cannot expect any considerable increase in our regular sources of income. Our present prosperity is therefore transient. During the next few years we must provide funds, in addition to the regular budget, to pay for foreign journals held in store for the duration, for the development of the Apparatus Department, including the services of a full-time mechanic, and especially for the building up of the Retirement Fund which is now far too small. The Executive Committee is considering the last item. The Committee on Additional Funds finds that the present time is not favorable for obtaining money, but it has laid plans for the future.

5. *Losses by Death*

In the death of Dr. Caswell Grave the Laboratory loses one of its most loyal members, whose important services, willingly given during a period of more than 30 years, are gratefully remembered.

Prof. A. D. Morrill, Emeritus Professor of Biology at Hamilton College, active in the early days of the Laboratory, died June 8, 1943.

Pof. John M. McFarlane, Emeritus Professor of Botany at the University of Pennsylvania, Trustee of the Corporation from 1897 to 1902, died September 16, 1943.

6. *Gifts*

The Laboratory is indebted to Mr. John Crane, for a letter written by his father, Charles R. Crane, to Mr. John D. Rockefeller, Jr., on the occasion of the setting up of the Endowment Fund to which both men had generously contributed. In the letter he refers in the following words to the unusual character of the Laboratory when he first become acquainted with it. "These scientists were struggling and accomplishing marvelous things with most meagre equipment, making many sacrifices. It seemed to me that the precious thing to preserve was the spirit of the organization, a spirit everywhere recognized although hard to seize or to imitate. So we have been most careful not in any way to jeopardize this spirit: and processes of organization and management have continued as I found them. It is a valuable expression of a democracy of educated, high minded men."

7. *Election of Trustees*

At the meeting of the Corporation held August 10, 1943, the following Trustees were elected Trustees Emeritus:

B. M. Duggar, The University of Wisconsin
W. E. Garrey, Vanderbilt University Medical School

The new Trustees elected at that meeting are:

Paul S. Galtsoff, U. S. Fish and Wild Life Service
E. W. Sinnott, Professor of Botany, Yale University

8. There are appended as parts of this report:

1. Appreciation of the Services of Professor Frank R. Lillie.
2. Memorials to deceased Trustees.
3. The Staff.
4. Investigators and Students, 1943.
5. A Tabular View of Attendance, 1939–1943.
6. Subscribing and Co-operating Institutions.
7. Evening Lectures.
8. Members of the Corporation.

Respectfully submitted,

CHARLES PACKARD,
Director

1. APPRECIATION OF THE SERVICES OF PROFESSOR FRANK R. LILLIE TO THE MARINE BIOLOGICAL LABORATORY

READ AT THE MEETING OF THE CORPORATION, AUGUST 10, 1943

In the history of the Marine Biological Laboratory the names of two men are pre-eminent: Dr. Whitman, who with prophetic insight, envisioned this institution as a national center of research in every department of Biology; and Dr. Lillie, who transformed that vision into reality. Coming to Woods Hole first in 1891 as an investigator receiving instruction, Dr. Lillie, with Dr. Whitman, organized the course in Embryology in 1893. He was appointed Assistant Director in 1900 at a time when the fortunes of the Laboratory were at a low ebb; Director in 1908; and President of the Corporation in 1926, after the successful conclusion of the campaign to obtain new buildings and an endowment. During the period from 1900 to 1942, when he resigned from the Presidency, the Marine Biological Laboratory developed from a struggling organization to its present position as the leading co-operative laboratory of the world.

It is of course true that only by the devoted work of the members of the Corporation, and the active interest of its many friends, could such an end be reached; but it is equally true that without wise guidance this effort would have failed. From the beginning, when Whitman, against every force and discouragement, fought for the principles of co-operation and independence, this Laboratory has pursued its steady course, adapting itself wisely to new conditions as they arose, but always holding to those basic ideals. During his fruitful years as Director, Dr. Lillie frequently stressed these principles. "Our purpose," he wrote, "is essentially ideal, and its pursuit demands our best efforts and our loyalty." And again, "We have laid the principle of co-operation at our foundation, and we have attempted to build it into every one of our activities." In this course he has always quietly led. There has never been any though of division since he has been in charge. Here lies his strength, and here lies the secret of the continued success of the Laboratory.

In accepting his resignation from the Presidency, the Corporation and the Trustees are rejoiced that he will continue his connection with the Laboratory as President Emeritus. We extend to him and to Mrs. Lillie, who has so ably assisted him in the development of the Marine Biological Laboratory, our grateful thanks, and we pledge to him our best efforts to continue the work which he has so long and so wisely guided.

C. E. McCLUNG
E. G. CONKLIN
CHARLES PACKARD

2. MEMORIALS TO DECEASED TRUSTEES

I. MEMORIAL TO DR. HERMON CAREY BUMPUS

Read by Dr. A. P. Mathews

Hermon Carey Bumpus, Trustee Emeritus of the Marine Biological Laboratory, died June 21, 1943, at the age of eighty-one years. The Laboratory thus loses a

member who played an active part in its development for more than forty years. Coming first to Woods Hole in 1889 when a graduate student of Whitman at Clark University, he worked here on his thesis, "The Embryology of the American Lobster." In 1890 he returned to Brown University, his Alma Mater, where he taught Comparative Anatomy for eleven years. It was during this period that he served at this Laboratory as head of the Invertebrate Zoology course; as Assistant Director from 1893 to 1895; and as Clerk of the Corporation from 1897 to 1899. He was a Trustee from 1897 to 1932 when he became Trustee Emeritus.

From 1898 to 1901 he was the Director of the Laboratory of the Fish Commission at Woods Hole, during which time he made a careful survey of the fauna of his region. At this time also he made one of the first studies of variation and its bearing on Natural Selection.

Many of his students from Brown came to us with him, among whom were George M. Gray, A. D. Mead, H. W. Walter, and F. P. Gorham.

Dr. Bumpus was remarkably efficient in the work of organization in all the positions which he held. At this Laboratory he greatly improved the equipment for work and for collecting living material; and due to his efforts the number of students at the Laboratory greatly increased, 85 being registered one year in the Invertebrate Course.

To bring Biology to the people was his chief interest. Accordingly he left Brown in 1901 to become Director of the American Museum of Natural History in New York, where he remained ten years and carried out his long cherished plans for taking the resources of the museum to the school children in New York City, an educational project which has since grown to great proportions throughout the country. Subsequently, while in the National Park Service, he developed many museums in the State and National Parks. In recognition of this work he received the Pugsley Medal for his service to education.

For three years he was Business Manager of the University of Wisconsin, and for five years, President of Tufts College. He was a Trustee also of several charitable institutions.

These are only a few of the many accomplishments of this tireless worker. He was, in the words of his student and life-long friend, Dr. H. E. Walter, "A natural teacher, and enthusiastic scientist, a remarkable executive, and a genial gentleman."

II. Memorial to Dr. G. N. Calkins

By Dr. L. L. Woodruff

The distinguished incumbent of the first Professorship of Protozoology in America, Gary Nathan Calkins, died at his home in Scarsdale, New York, on January 4, 1943, after a considerable period of ill health which was endured with characteristic cheerfulness and fortitude.

Calkins was born at Valparaiso, Indiana, on January 18, 1869, but spent nearly all of his life on the Eastern seaboard. His scientific training began at the Massachusetts Institute of Technology where, under the influence of Professor William T. Sedgwick, an interest was aroused in biology as a profession. After receiving the B.S. degree in 1890 he served until 1893 as lecturer at the Institute and also as Assistant Biologist to the Massachusetts State Board of Health. Then he trans-

ferred to Columbia University to study under Professor Edmund D. Wilson and received the Ph.D. degree in 1898. While a graduate student he was appointed to the teaching staff and thus began the life-long membership in the Department of Zoology at Columbia, which in 1907 culminated as Professor of Protozoology. Calkins was for some years the Executive Officer of the Department, and retired in 1940 as Professor Emeritus in residence. Columbia University conferred on him the honorary degree of Sc.D. in 1929.

Calkins' devotion of his life to the study of the Protozoa was inspired both by an inherent interest in the "little animals," and the well-founded belief that they afford highly favorable material for the approach to many general biological problems. This is best exemplified by his most important treatise, *The Biology of the Protozoa* (1926, 2nd edition 1933), and his long-continued studies on the physiology and cytology of free-living Ciliates, with particular reference to the significance of fertilization and other factors influencing longevity. In this classic work he devised more exact methods of pedigreed culture, involving daily isolation of the animals, that laid the foundations for present-day technique in the field, and he also developed what may be referred to as his philosophy of the Protozoan individual. Both phases stimulated many investigators to enter similar fields.

The extensive series of important studies from Calkins' laboratory is but one of his many contributions to science. A brilliant lecturer and teacher at Columbia and at The Marine Biological Laboratory, his courses revealed a comprehensive grasp of protozoology from both its theoretical and practical aspects that inspired many students; and his versatility was shown by numerous other activities. Thus, for example, he was Consulting Biologist to the New York State Cancer Laboratory at Buffalo from 1902 to 1908, Lecturer before the Lowell Institute in 1907, President of the Association for Cancer Research in 1916, President of the Society for Experimental Biology and Medicine from 1919 to 1921, and Director of the University Union in Paris in 1926 and 1927. He was elected in 1919 to the National Academy of Sciences.

Calkins' association with The Marine Biological Laboratory began just over a half century ago, and for about forty years he was in regular attendance as an investigator. He was a member of the Corporation for 39 years and its Clerk for 17 years, member of the Board of Trustees for 30 years and its Secretary for 12 years, member of the Research Staff for 31 years, and head of the Protozoology course, which he founded, for 22 years.

Zoology in general and Columbia University and The Marine Biological Laboratory in particular owe to Calkins more than can be readily expressed for his scientific contributions, teaching, and administrative service. All this, as well as his personal charm, unfailing enthusiasm, and hearty good fellowship, was attested by his former students and associates who presented to him after retirement a volume of nearly two hundred letters of esteem and appreciation inscribed:

Gary Nathan Calkins
Philosopher in Little Things
and Friend.

3. THE STAFF, 1943

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

E. G. CONKLIN, Professor of Zoology, Emeritus, Princeton University.
CASWELL GRAVE, Professor of Zoology, Emeritus, Washington University.
FRANK R. LILLIE, Professor of Embryology, Emeritus, The University of Chicago.
RALPH S. LILLIE, Professor of General Physiology, Emeritus, The University of Chicago.
C. E. MCCLUNG, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. MAST, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. MATHEWS, Professor of Biochemistry, Emeritus, University of Cincinnati.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. CONSULTANTS

T. H. BISSONNETTE, Professor of Biology, Trinity College.
L. L. WOODRUFF, Professor of Protozoology, Yale University.

II. INSTRUCTORS

J. B. BUCK, Assistant Professor of Zoology, University of Rochester, in charge of course.
W. D. BURBANCK, Associate Professor of Biology, Drury College.
M. D. BURKENROAD, Assistant Curator, Bingham Oceanographic Foundation, Yale University.
C. G. GOODCHILD, Associate Professor of Biology, Southwest Missouri State Teachers College.
RONALD GRANT, Lecturer in Physiology, McGill University.
JOHN H. LOCHHEAD, Instructor in Zoology, University of Vermont.
MADELENE E. PIERCE, Assistant Professor of Zoology, Vassar College.
MARY D. ROGICK, Professor of Biology, College of New Rochelle.

III. LABORATORY ASSISTANT

MARGARET L. KEISTER, Instructor in Biology, Wheaton College.

EMBRYOLOGY

I. CONSULTANTS

L. G. BARTH, Assistant Professor of Zoology, Columbia University.
H. B. GOODRICH, Professor of Biology, Wesleyan University.

II. INSTRUCTORS

VIKTOR HAMBURGER, Professor of Zoology, Washington University, in charge of course.
DONALD P. COSTELLO, Assistant Professor of Zoology, University of North Carolina.
CHARLES B. METZ, Instructor, Wesleyan University.

PHYSIOLOGY

I. CONSULTANTS

WILLIAM R. AMBERSON, Professor of Physiology, University of Maryland, School of Medicine.

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Vanderbilt University Medical School.
MERKEL H. JACOBS, Professor of Physiology, University of Pennsylvania.

II. INSTRUCTORS

ARTHUR K. PARPART, Associate Professor of Biology, Princeton University, in charge of course.
ROBERT BALLENTINE, Lecturer in Zoology, Columbia University (absent in 1943).
KENNETH C. FISHER, Assistant Professor of Experimental Biology, University of Toronto.
ARTHUR C. GIESE, Associate Professor of Biology, Stanford University (absent in 1943).
RUDOLF T. KEMPTON, Professor of Zoology, Vassar College.
F. J. M. SICHEL, Assistant Professor of Physiology, University of Vermont, College of Medicine.

BOTANY

I. CONSULTANTS

S. C. BROOKS, Professor of Zoology, University of California.
B. M. DUGGAR, Professor of Plant Physiology, University of Wisconsin.

II. INSTRUCTORS

WM. RANDOLPH TAYLOR, Professor of Botany, University of Michigan, in charge of course.
HANNAH CROASDALE, Technical Assistant, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. FAILLA, Memorial Hospital, New York City.
L. ROBINSON HYDE, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

PRISCILLA B. MONTGOMERY (MRS. THOMAS H. MONTGOMERY, JR.), Librarian
DEBORAH LAWRENCE MARY A. ROHAN S. MABELL THOMBS

APPARATUS DEPARTMENT

E. P. LITTLE, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. GRAHAM R. S. LILJESTRAND

CHEMICAL DEPARTMENT

E. P. LITTLE, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

JAMES MCINNIS, Manager
RUTH CROWELL BERNICE HALL
M. B. GRAY W. E. KAHLER G. LEHY
A. M. HILTON A. W. LEATHERS F. N. WHITMAN

GENERAL OFFICE

F. M. MACNAUGHT, Business Manager
POLLY L. CROWELL ANITA GIBSON

GENERAL MAINTENANCE

T. E. LARKIN, Superintendent

W. C. HEMENWAY — T. E. TAWELL
R. W. KAHLER — J. WYNNE
A. J. PIERCE

THE GEORGE M. GRAY MUSEUM

GEORGE M. GRAY, Curator Emeritus

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4. INVESTIGATORS AND STUDENTS

Independent Investigators, 1943

ADDISON, WILLIAM H. F., Professor of Normal Histology and Embryology, University of Pennsylvania, School of Medicine.
BALL, ERIC G., Associate Professor of Biological Chemistry, Harvard Medical School.
BARTH, L. G., Assistant Professor of Zoology, Columbia University.
BERGER, CHARLES A., Director, Biological Laboratory, Fordham University.
BROOKS, MATILDA M., Research Associate in Biology, University of California.
BROWN, DUGALD E. S., Professor of Physiology, New York University, College of Dentistry.
BUCK, JOHN B., Assistant Professor of Zoology, University of Rochester.
BUDINGTON, ROBERT A., Professor of Zoology, Emeritus, Oberlin College.
BUGGS, CHARLES W., Professor of Biology and Head of Division of the Sciences, Dillard University.
BURBANCK, WILLIAM D., Associate Professor and Chairman of Department of Biology, Drury College.
BURKENROAD, MARTIN D., Assistant Curator, Bingham Oceanographic Foundation, Yale University.
CHAMBERS, ROBERT, Research Professor of Biology, Washington Square College, New York University.
CLARK, ELEANOR L., Voluntary Investigator, University of Pennsylvania, School of Medicine.
CLARK, E. R., Professor and Head of Department of Anatomy, University of Pennsylvania, School of Medicine.
CLEMENT, ANTHONY C., Associate Professor of Biology, College of Charleston.
CLOWES, G. H. A., Director of Research, Eli Lilly and Company.
CONKLIN, EDWIN G., Professor of Biology, Emeritus, Princeton University.
COSTELLO, DONALD P., Associate Professor of Zoology, University of North Carolina.
DUGGAR, BENJAMIN M., Professor of Plant Physiology, University of Wisconsin.
EVANS, TITUS C., Assistant Professor of Radiology, College of Physicians and Surgeons, Columbia University.
FAILLA, G., Professor of Radiology, College of Physicians and Surgeons, Columbia University.
FISHER, KENNETH C., Assistant Professor of Physiological Zoology, University of Toronto.
GABRIEL, MORDECAI L., Department of Zoology, Columbia University.
GALTSOFF, PAUL S., Senior Biologist, U. S. Fish and Wildlife Service.
GARREY, W. E., Professor of Physiology, Vanderbilt University, School of Medicine.
GLASER, OTTO C., Professor of Biology, Amherst College.
GOODCHILD, CHAUNCEY G., Associate Professor of Biology, State Teachers College, Springfield, Missouri.
GRAND, C. G., Research Associate, Washington Square College, New York University.
GRANICK, SAM, Assistant, Rockefeller Institute.
GRANT, RONALD, Lecturer in Zoology and Physiology, McGill University.
GRAVE, CASWELL, Professor of Zoology, Emeritus, Washington University.

HAMBURGER, VIKTOR, Professor of Zoology, Washington University.
HARNLY, MORRIS H., Associate Professor of Biology, Washington Square College, New York University.
HARRIS, DANIEL L., Research Associate, University of Pennsylvania.
HARTMAN, FRANK A., Professor and Chairman of Department of Physiology, Ohio State University.
HARVEY, ETHEL B., Independent Investigator in Biology, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HEILBRUNN, L. V., Associate Professor of Zoology, University of Pennsylvania.
HIATT, EDWIN P., Assistant Professor, New York University, College of Dentistry.
HOPKINS, HOYT S., Associate Professor of Physiology, New York University, College of Dentistry.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania, School of Medicine.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KRAHL, M. E., Chemist, Eli Lilly and Company.
LAVIN, GEORGE, Rockefeller Institute for Medical Research.
LEE, RICHARD E., Research Associate, Washington Square College, New York University.
LILLIE, FRANK R., Professor of Zoology, Emeritus, The University of Chicago.
LILLIE, RALPH S., Professor of Physiology, Emeritus, The University of Chicago.
LITTLE, E. P., Instructor in Science, Phillips Exeter Academy.
LOCHHEAD, JOHN H., Instructor in Zoology, University of Vermont.
MCCLUNG, C. E., Professor of Zoology, Emeritus, University of Pennsylvania.
MCELROY, WILLIAM D., Research Associate, Princeton University.
MARSLAND, DOUGLAS A., Associate Professor of Biology, Washington Square College, New York University.
MAST, S. O., Professor of Zoology, Emeritus, Johns Hopkins University.
MATHEWS, ALBERT P., Professor of Biochemistry, Emeritus, University of Cincinnati.
MEGLITSCH, PAUL, Head of Department of Zoology, Herzl Junior College.
MEMHARD, ALLEN R., Crescent Road, Riverside, Connecticut.
METZ, CHARLES B., Instructor in Biology, Wesleyan University.
MICHAELIS, LEONOR, Rockefeller Institute for Medical Research.
MORGAN, LILLIAN V., California Institute of Technology.
MORGAN, T. H., Professor of Biology, California Institute of Technology.
NELSON, LEONARD, Medical Student, University of Pennsylvania.
OSTERHOUT, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
PACKARD, CHARLES, Director, Marine Biological Laboratory.
PARKER, GEORGE H., Professor of Zoology, Emeritus, Harvard University.
PARMENTER, CHARLES L., Professor of Zoology, University of Pennsylvania.
PARPART, A. R., Associate Professor of Biology, Princeton University.
PIERCE, MADELENE E., Assistant Professor of Zoology, Vassar College.
POLLISTER, ARTHUR W., Associate Professor of Zoology, Columbia University.
REINHARD, EDWARD G., Head, Department of Biology, Catholic University of America.
ROGICK, MARY D., Professor of Biology, College of New Rochelle.
ROSE, S. MERYL, Instructor in Zoology, Smith College.
RUSSELL, ALICE M., Instructor in Zoology, Fordham University.
SCHAEFFER, A. A., Professor of Biology, Temple University.
SCHALLEK, WILLIAM B., Fellow, Woods Hole Oceanographic Institution.
SCOTT, SISTER FLORENCE M., Professor of Biology, Seton Hill College.
SICHEL, ELSA K., Head of Science Department, Vermont State Normal School.
SICHEL, F. J. M., Assistant Professor of Physiology, University of Vermont College of Medicine.
SLIFER, ELEANOR H., Assistant Professor of Zoology, State University of Iowa.
SOSA, JULIO M., Professor of Histology and Embryology, College of Medicine, Montevideo, Uruguay.
STEINBACH, H. BURR, Associate Professor of Zoology, Washington University.
STOKEY, ALMA G., Professor of Botany, Emeritus, Mount Holyoke College.

STUNKARD, HORACE W., Professor of Biology, New York University.
TAYLOR, WILLIAM R., Professor of Botany, University of Michigan.
WENRICH, D. H., Professor of Zoology, University of Pennsylvania.
WHITING, ANNA R., Guest Investigator, University of Pennsylvania.
WHITING, P. W., Associate Professor of Zoology, University of Pennsylvania.
WIERCINSKI, FLOYD J., Instructor in Biology, Catholic University of America.
WRINCH, DOROTHY, Research Associate in Physics, Smith College.
ZWEIFACH, BENJAMIN W., Research Associate in Biology, Washington Square College, New York University.

Research Assistants, 1943

ABRAMSKY, TESSIE, Technician, Rockefeller Institute for Medical Research.
BEHAN, ANNE, Research Assistant, Columbia University.
GIDGE, NATALIE, Smith College.
HEIDENTHAL, GERTRUDE, Research Assistant, University of Pennsylvania.
HONEGGER, CAROL, Research Assistant, Temple University.
HUTCHINSON, DOROTHEA, Cazenovia Junior College.
JOHN, HEDDA MARIA, Research Assistant in Neurology, Columbia University.
KAWATA, NOBUYUKI, Research Assistant, Washington University.
KRUGELIS, EDITH, Research Assistant, Columbia University.
LAWLER, HELEN CLAIRE, Research Assistant, New York University.
LOWENHAUPT, MARIAN, Graduate Student, Washington University.
MINER, KARYL R., New York University.
ODLAUG, T. O., Research Biologist, Fish and Wildlife Service.
QUINN, GERTRUDE PATRICIA, Research Assistant, New York University, College of Dentistry.
STERN, JOSEPH R., University of Toronto.
WOODWARD, ARTHUR A., JR., Research Assistant, University of Pennsylvania.
YARNALL, MARGARET, Student, University of Pennsylvania.

Library Readers, 1943

AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland Medical School.
ANDERSON, THOMAS F., Associate, Johnson Foundation, University of Pennsylvania.
BECK, LYLE V., Instructor in Physiology, Hahnemann Medical College.
BOCHE, ROBERT D., Instructor in Zoology, University of Pennsylvania.
BOTSFORD, E. FRANCES, Associate Professor of Zoology, Connecticut College.
BRODIE, BERNARD B., Research Associate in Biochemistry, New York University.
BROWNELL, KATHARINE A., Research Associate in Physiology, Ohio State University.
CAHEN, RAYMOND L., Research Assistant in Pharmacology, Yale University Medical School.
CAHN, T., New York University.
CAHNMANN, HANS J., Research Associate, Mt. Sinai Hospital.
CASSIDY, HAROLD G., Instructor in Organic Chemistry, Yale University.
CHIDSEY, JANE L., Assistant Professor of Zoology, Wheaton College.
CROASDALE, HANNAH T., Technical Assistant, Dartmouth College.
FRANKLIN, ROGER G., Professor of Biology, St. Joseph's Seminary.
FURTH, JACOB, Associate Professor of Pathology, Cornell University Medical College.
GATES, R. RUGGLES, Professor of Botany, Emeritus, University of London.
GUREWICH, VLADIMIR, Clinical Assistant and Attending Physician, Cornell Division of the Bellevue Hospital.
HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
HIBBARD, HOPE, Professor of Zoology, Oberlin College.
KRASNOW, FRANCES, Head, Department of Biochemistry, Guggenheim Dental Clinic.
LILLY, JOHN C., Fellow in Biophysics, University of Pennsylvania.
LOWENSTEIN, OTTO, Clinical Professor of Neurology, New York University.
LOEWI, OTTO, Research Professor, New York University, College of Medicine.
MARSHALL, HELEN A., Massachusetts State College.
MAYOR, JAMES W., Professor of Biology, Union College.

MENKIN, VALY, Assistant Professor of Pathology, Harvard Medical School.
MEYERHOF, OTTO, Research Professor of Biochemistry, University of Pennsylvania.
MOLDAVER, JOSEPH, Research Assistant in Neurology, Columbia University.
NACHMANSOHN, DAVID, Research Associate in Neurology, Columbia University.
SCHMITT, FRANCIS O., Head, Department of Biology and Biological Engineering, Massachusetts Institute of Technology.
SILOW, RONALD A., Geneticist, Cotton Research Station, Trinidad.
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SATAKE, JACK, Graduate Student, Washington University.
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Students, 1943

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COLE, ELSIE L., Graduate Teaching Assistant, University of Wisconsin.
HOPKINS, ALICE, Student, University of Rochester.
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Wood, Marcia, Russell Sage College.

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Brooks, Edith H., Student, Swarthmore College.
Chapman, Mary P., Student, Vassar College.
Chew, Robert M., Student, Washington and Jefferson College.
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Holloway, Rita H., Student, Oberlin College.
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Jan Tausch, Anne M., Student, New Jersey College for Women.
Jensen, Dorothy, Mount Holyoke College.
Katsafanas, Georgia, Student, Seton Hill College.
Kelley, Ellen M., Student, New Jersey College for Women.
Justitia, Sister Mary, Student, Fordham University.
Lieb, Margaret, Student, Smith College.
Marsh, Mary G., Vassar College.
Murray, Helen E., Student, Emmanuel College.
Paisley, Anne, Student, DePauw University.
Pope, Edith, Student, Smith College.
Pope, Louise S., Instructor in Biology, Whitman College.
Pope, Philip H., Professor of Biology, Whitman College.
Ransom, Gladys Virginia, Biology Laboratory Assistant, Wilson College.
Reich, Eva, Student, Barnard College.
Richards, Philip W., Groton School.
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Rubright, Eleanor, Student, Ohio Wesleyan University.
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Taylor, Ruth M., Student, Oberlin College.
Uphoff, Delta E., Student, Russell Sage College.
Weber, Kathleen K., Student, University of Richmond.
Williams, Patricia A., Student, Seton Hill College.
Witkus, Eleanor R., Research Associate, Fordham University.
Yamamato, Kekahuna H., DePauw University.
Zarudnaya, Katerina I., Graduate Assistant, Johns Hopkins University.

5. TABULAR VIEW OF ATTENDANCE

	1939	*1940*	*1941*	*1942*	*1943*
INVESTIGATORS—Total	352	386	337	201	160
Independent	213	253	197	132	89
Under instruction	60	62	59	16	19
Research assistants	79	71	50	25	17
Library readers	—	—	31	28	35
STUDENTS—Total	133	128	131	74	68
Zoology	55	55	55	36	47
Protozoology (not given after 1940)	12	7	—	—	—
Embryology	36	34	37	24	13
Physiology	21	22	24	6	8
Botany	9	10	15	8	—
TOTAL ATTENDANCE	485	514	468	275	228
Less persons registered as both students and investigators	14	7	7	2	6
	471	507	461	273	222
INSTITUTIONS REPRESENTED—Total	162	148	144	126	116
By investigators	132	112	102	83	70
By students	72	79	72	43	41
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	2	1	5	2	2
By students	2	2	2	–	1
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	8	2	3	–	2
By students	1	1	1	–	–

6. SUBSCRIBING AND COOPERATING INSTITUTIONS

1943

Amherst College
Atlanta University
Bell Telephone Laboratories, Inc.
Biological Institute, Philadelphia, Pennsylvania
Bowdoin College
Brooklyn College
Bryn Mawr College
Catholic University of America
College of Physicians and Surgeons
Columbia University
Commonwealth Fund
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Dept. of the Interior
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Eli Lilly & Co.
Massachusetts General Hospital
Mount Holyoke College
New York University
New York University College of Medicine
New York University Washington Square College
Oberlin College
Ohio State University
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Institute for Medical Research
Rutgers University
Seton Hill College
Smith College
State University of Iowa
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Pennsylvania
University of Pennsylvania School of Medicine

University of Pennsylvania Johnson Foundation
University of Rochester
Vassar College
Villanova College
Washington University
Wellesley College
Wheaton College
Woods Hole Oceanographic Institution
Yale University

7. EVENING LECTURES, 1943

Friday, July 9
Dr. W. R. Taylor ..... "Utilization of Marine Plants."
Friday, July 16
Dr. D. P. Costello ..... "Experiments on the Localization of Developmental Factors in the Egg of Nereis."
Friday, July 23
Dr. Paul S. Galtsoff ..... "Physiology of Sex and Sex Change in the Genus Ostrea."
Friday, July 30
Dr. Rudolf T. Kempton ..... "Renal Secretion."
Friday, August 6
Dr. L. V. Heilbrunn ..... "The Calcium-Release Theory of Stimulation."
Friday, August 13
Dr. Kurt G. Stern ..... "Studies on Iron Proteins."
Friday, August 20
Dr. B. M. Duggar ..... "Studies in the Irradiation of Certain Microorganisms."
Friday, August 27
Mr. A. H. Woodcock ..... "Wind-induced Motion of the Physalia."

8. MEMBERS OF THE CORPORATION, 1943

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
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EMBRYONIC GROWTH IN THE VIVIPAROUS POECILIID, HETERANDRIA FORMOSA[1]

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In *Heterandria formosa* the fertilized eggs are minute and the embryos secure their nourishment for development through a placental type of association with the mother. This paper presents an analysis of embryonic growth in this viviparous species and a comparison of this growth with that in oviparous fishes. In the discussion an attempt is made to evaluate the factors restricting growth in Heterandria. A similar study of embryonic growth in a number of ovoviviparous fishes is to follow.

Bailey (1933) and Turner (1937) have directed attention to the development of embryos in poeciliid fishes in relation to the mother. Turner (1940a, b, c, d) surveyed the various types of association between mother and embryo for the four viviparous Cyprinodont families (Poeciliidae, Anablepidae, Goodeidae, and Jenynsiidae).

For the oviparous fishes, the work of Gray (1926, 1928) on the development of the trout *Salmo fario* stands almost alone. However, Kronfeld and Scheminzki (1926) have also contributed data on the trout. This work on a fish species totally dependent upon yolk for its embryonic nourishment provides the basis for the comparison and interpretation of many of the observations described below.

Material

Heterandria formosa is remarkable in showing a high degree of superfetation. As many as eight broods of developing embryos occur within the ovary of a single female. Active sperm are retained in the ovary for many months following a single copulation. Thus it is impossible to determine the exact time of fertilization or the time it has taken any particular brood to reach the stage in which it is found.

Its embryonic development resembles in its general aspects that of Fundulus and other Cyprinodont fishes. In Heterandria, as in all poeciliid fishes, the embryos are retained until parturition within the follicles of the single median ovary. Following fertilization the ovum and the follicle become separated by the perivitelline space and fluid. The follicular membrane is henceforth generally referred to as the ovisac. Its diameter increases with the extension of the fluid filled spaces associated with the embryo and with the growth of the embryo itself.

Sections of the immature ova show little yolk, but numerous small oil globules are already present (Fig. 1). The latter increase in volume as the eggs grow, and their number is reduced by the coalescence of the small vacuoles (Fig. 2). The large oil globules fuse at the time of fertilization to form a single large spherical oil

[1] The author expresses appreciation to Dr. Leigh Hoadley for suggestions and encouragement in this work.

mass which occupies from 50 to 75 per cent of the volume of the ripe ovum (Fig. 3). When the blastodisc appears, it bears the same relation to the oil globule in Heterandria as it does to the yolk mass in the more typical teleost egg. Thus a central periblast is observed beneath the blastodisc and a thin syncytial periblast eventually surrounds the oil globule in precisely the same manner as it surrounds the yolk in Fundulus.

The chemical composition of the oil globule has not been determined, but it stains deeply with the fat dyes Sharlach R and Sudan III. The great reduction in the amount of the other yolk components present would suggest that the food of the embryo during the first part of its development might be derived from the oil. Figure 4 shows the relation between the diameter of the ovisac and the volume of

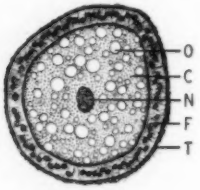

FIGURE 1

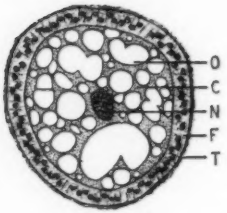

FIGURE 2

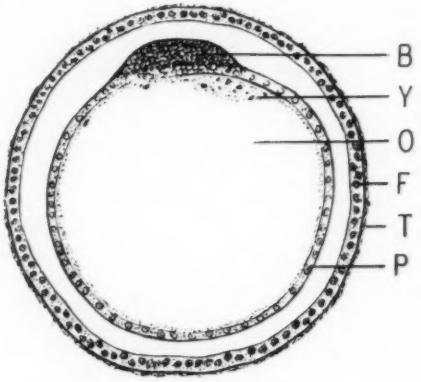

FIGURE 3

FIGURE 1. Early ovum of *Heterandria formosa*. O, oil; C, cytoplasm; N, nucleus; F, follicular epithelium; T, theca.

FIGURE 2. Late ovum of *Heterandria formosa*.

FIGURE 3. Early blastodisc of *Heterandria formosa*. B, blastodisc; Y, yolky periblast; Ch, chorion.

the oil globule. It indicates that the volume is decreased by less than 30 per cent up to the time that the pseudo-placenta is fully formed.

Fraser and Renton (1940) and Turner (1940a) have described the fetal adaptations associated with viviparity in Heterandria. These are unique and may be summarized as follows: The antero-ventral somatopleure of the embryo becomes enormously extended and encloses the head completely. This produces a large extraembryonic pericardial cavity extending into the head fold. The postero-ventral somatopleure is also somewhat expanded and contains the liver, gut and the much extended urinary bladder. An extensive capillary network develops on this expanded portion of the somatopleure so that its whole surface opposing the follicular epithelium is highly vascularized. Since the latter has likewise developed an extensive vascular system, an association between embryonic and maternal circulation comparable to a non-deciduate mammalian placenta is formed.

In relatively advanced embryos the pericardial hood ruptures over the forepart of the head, leaving a strap of somatopleure over it. This strap gradually decreases in size and eventually disappears. As a result the area of the vascular association between embryo and ovisac has been considerably reduced by the time of parturition.

Special types of cells in connection with the maternal and embryonic components of the pseudo-placenta also appear. Turner (1940a) has stated that the outer layer of the extra-embryonic pericardial cavity, the ectoderm, is equipped with "conical cells, which are granular at the base and vacuolated apically . . . and sometimes gathered into low mounds." Their role has not been determined but it

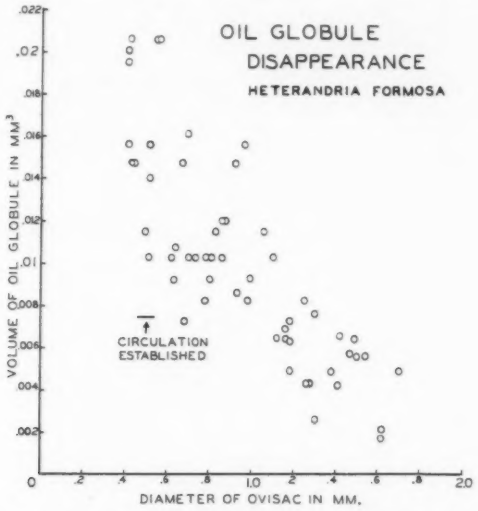

FIGURE 4. The volume in cubic millimeters of the oil globule in fertilized eggs and embryos of Heterandria is plotted as a function of the diameter of the ovisac in millimeters. Each point represents a single pair of readings.

is assumed that they aid in some way the nourishment of the embryo through the pseudo-placenta.

Another cell specialization has not been described previously but seems to become important about the time that the head breaks through the enveloping pericardial sac. It probably represents a supplementary nutritive mechanism which helps to compensate for the reduction in association surface. Sometime during the last quarter of development, the wall of the ovisac, which has appeared as a squamous epithelium since fertilization, hypertrophies. In a restricted region of the ovisac prominent secretory cells are developed. These seem to form an approximately one-third of the surface of the ovisac and parallel more or less closely the embryonic vascular surface. In Figure 5 these cells are seen opposite the blood vessels in the wall of the pericardium, but they may also occur opposite nonvascularized body epithelium. Histologically they appear to be active secretory cells

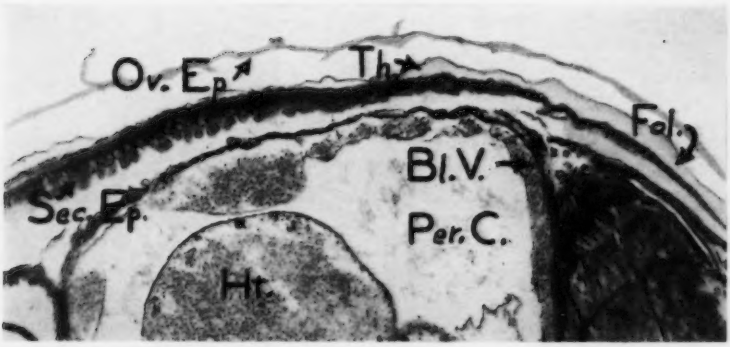

FIGURE 5. Photomicrograph of a section through the ovisac and pericardium of a later embryo of *Heterandria formosa* showing secretory cells of the ovisac epithelium. Ov. Ep., ovarian epithelium; Th., theca; Fol., follicular epithelium; Sec. Ep., secretory portion of follicular epithelium; Bl. V., blood vessel; Per. Cav., pericardial cavity; Ht., heart.

containing many secretion granules. Coagulum which may be from this source appears in sections through the cavity of the ovisac and also in the gut.

METHODS

Both wet and dry weights were determined for members of each brood of embryos taken from 16 females with varied previous histories. One hundred and fifty-one embryos were weighed either individually or in groups belonging to the same brood. The embryos were dissected from the ovary with the ovisac intact. The diameter[2] of the ovisac was measured with an ocular micrometer. The embryos were allowed to touch fine absorbent paper to remove surplus fluid and immediately placed on flamed platinum squares of known weight. These were weighed at once in a single rapid trial to determine the wet weight of the embryo in the intact ovisac. They were then dried over calcium chloride to constant weight.

All weighings were made with a Sartorius balance. The initial weights of the platinum squares and the final weights of the squares plus dried embryos were each determined by 10 to 20 readings (with zero correction before and after each reading). The standard deviation[3] for a given series of readings was rarely over .05 mg. In several trial cases two weeks in the dessicator intervened between the first and second group of ten readings. The standard deviation was still below .05 mg.

The smaller embryos were weighed in groups. The average wet weight of 30 of the earliest embryos was 0.026 mg. After a consideration of all sources of error,

[2] The ovisac is spherical at first but becomes increasingly ellipsoidal as development progresses. In the late stages the average of several measurements made on the same ovisac proved to be a reasonably consistent measure of size and was recorded as if it were a true diameter.

[3] $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$.

this value was estimated to be within at least 0.008 mg. of the true wet weight at the time of fertilization. The dry weight value of 0.017 mgs. was similarly estimated to be within at least 0.002 mgs. of the correct value for this stage. In all of the weight measurements except those on the very early stages the experimental error was small compared to the normal variation of the material.

Ash determinations are impractical for early stages because of the extremely small weights involved. Later stages were ashed in a small muffle furnace and an average ash content of 2 to 3 per cent of the dry weight found.

Weight Changes of the Developing Embryo

Since the age of the embryos was not known, the diameter of the ovisac was selected as a convenient indication of the stage of development, and the weight determinations were correlated with this dimension. When this was done the great increase in weight of the embryo in the intact ovisac was apparent (Fig. 6). The relationship between dry weight and ovisac diameter is repeated on a larger scale for later comparisons (Fig. 7).

Before any appreciable gain in dry weight can be detected the wet weight can be seen to have increased markedly. In fact, the water content of the whole egg

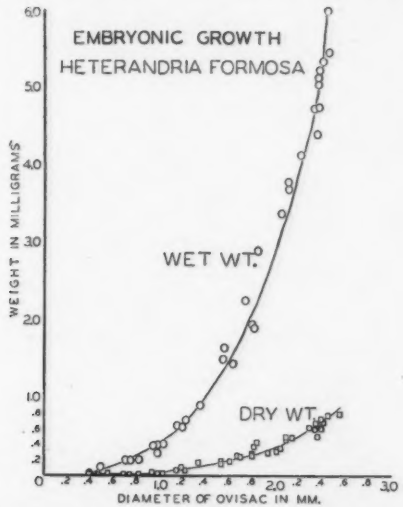

Figure 6. The curves show the increase in both wet and dry weights of developing embryos of *Heterandria formosa* plotted as a function of the diameter of the ovisac, a convenient measure of development.

plus ovisac increases from 34.6 per cent to 86.8 per cent in this period. There is considerable experimental error in the first figure because of the small weights involved, but a value of 35 per cent is consistent with the large amount of oil present.

After the dry weight has begun to increase, the relation between wet and dry weight stays remarkably constant. When the period was divided into two sub-

periods, the first including embryos with ovisac diameters between 1.00 and 2.00 and the second including embryos with ovisac diameters greater than 2.00, the differences in per cent dry weight were not significant. During the first sub-period the dry weight average 12.84 per cent of the wet weight (σ 1.8%) and during the second it was 13.49 per cent (σ 1.9%). The average was 13.2 per cent.

After parturition the embryo breaks out of the fluid-filled ovisac and the extra liquid in the expanded pericardial cavity disappears. Accordingly the percentage dry weight increases abruptly to a stable value for the young fish of 20.6 per cent (σ 1.7%). This information can be diagrammed to show the changes in composition from the egg at the time of fertilization to the larva at the time of parturition (Fig. 8).

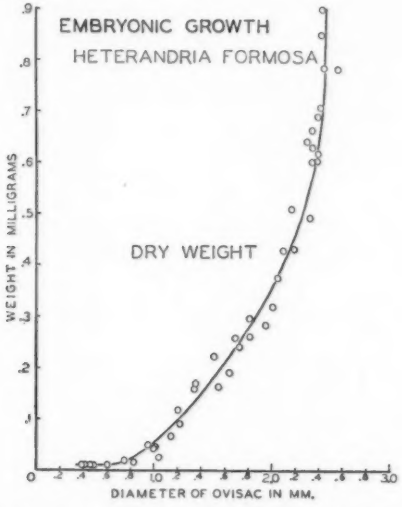

FIGURE 7. The curve shows the increase in the dry weight of embryos of *Heterandria formosa* plotted as a function of the diameter of the ovisac. This represents the same weight values shown in the dry weight curve of Figure 6, but the units of the weight ordinate are much expanded.

These changes can also be represented in the form of a generalized equation which shows the relation of .1 mg. of ovum to the final weight of the larva.

0.1 mg.	+	2.6 mg.	+	15.8 mg.	=	18.5 mg.
(wet weight of egg)		(dry weight from mother)		(wet weight from mother		(wet weight of young fish)

This equation expresses the overall changes. A similar expression has been developed for the trout by Gray (1926). His formula holds from the time the embryo is 50 per cent developed to the time it is 80 per cent formed during which time it has increased about 400 per cent in weight. During this period the trout embryo converts 1.0 grams of yolk (wet weight) plus 0.7 grams of water into 1.56 grams of fish.

Since the yolk makes up almost the entire egg, it can be estimated that one gram of trout egg makes only slightly more than 1.5 grams of trout embryo. In comparison one gram of Heterandria egg would result in 185 grams of embryo. This strikingly illustrates the importance of the maternal contribution of nourishment in this species.

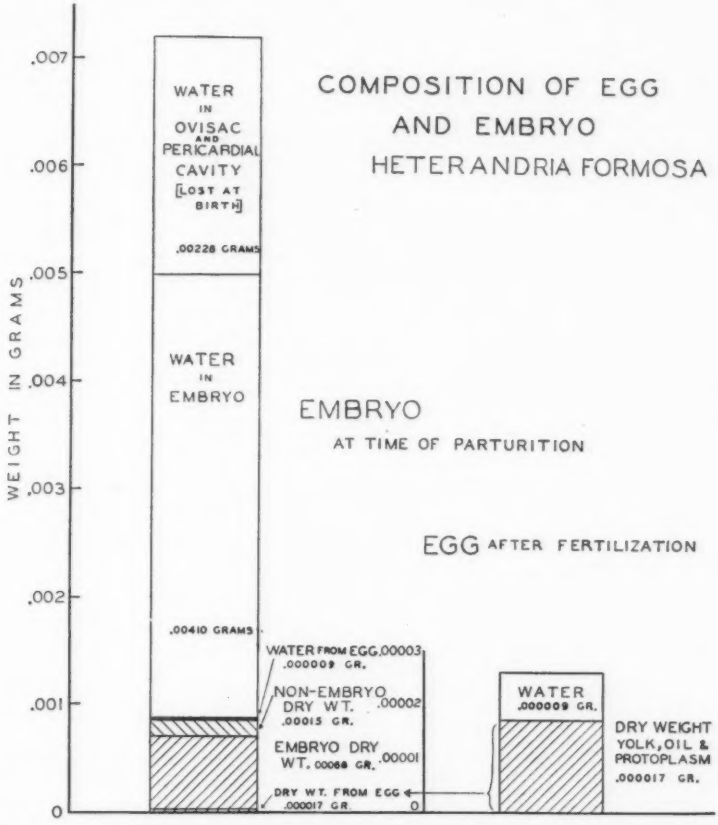

FIGURE 8. The composition of a typical embryo ready for parturition and of a recently fertilized egg is diagrammed. Wide variations from the actual figures cited were found but the proportions remained generally the same in the various larvae studied. As discussed in the text, the weight figures and the relative amounts of wet and dry material in the fertilized egg have been determined only approximately.

In the above figures for the trout the discrepancy of .14 grams between the 1.7 grams of yolk and water and the resulting 1.56 gram weight of the larva represents the dry weight used for maintenance metabolism. The dry weight used for maintenance in Heterandria could not be measured directly. However, it can be as-

sumed that approximately one-third of the total food available is used for the maintenance of the embryo in Heterandria as well as in the trout.[4] The maintenance dry weight calculated in this way can be introduced into an equation expressing the actual dry weight drain on the mother per embryo.

water	+	6.8 mg. (dry weight of embryo)	+	2.2 mg. (maintenance dry weight)	=	9.0 mg. (total dry weight from mother)	+	water

Growth Rate of the Developing Embryo

Thus far the magnitude of the embryonic weight increase has been described, but no indication of the rate has been obtained. Observations of living gravid females suggested a method whereby a time axis might be obtained. The young of females kept under constant conditions showed a tendency to be born at regular intervals.[5] Accordingly, the weights of all the broods of a single female kept under relatively constant conditions were plotted as if the time intervals between them were the same. This treatment yielded consistent and apparently significant growth curves.

The female whose embryos are presented in Figure 9 has the unusually large number of eight broods. The growth relationships believed to be general for Heterandria are therefore well shown. The initial portion of the curve is a straight line which appears to be parallel to the time axis. This line represents the early developmental period when no post-fertilization contribution of nourishment from the mother can be detected. The second part of the curve is a steeply ascending straight line. It extends throughout the greater part of the embryonic period and represents an approximately constant growth increment in dry weight contributed by the mother. Obviously, there must be a transitional period, which the data cannot show, during which the pseudo-placental associations are being established. This has been indicated by the dotted lines in Figure 9 and also in Figure 10 which represents the similar treatment of embryo weights obtained in the study of other females. All of the females which had been kept under relatively constant conditions showed this relationship. Unfortunately, the concept cannot be tested for females with fewer than five broods as can be seen from the number of points required to establish the two straight lines in Figures 9 and 10.

In order to extend the study to more females, records of ovaries dissected over a three year period were re-examined. These records gave the number of broods per female together with their average ovisac diameters for fish kept under a variety of experimental conditions. The average weight of the broods in these females

[4] The ratio between the dry weight of the embryo and the dry weight of nutriment required to produce this quantity of embryo is the efficiency coefficient of development. Gray reports a value of .65 for the trout and points out that this figure has been found to be approximately the same for a wide variety of organisms (cf. Murray, 1926, chick; Hayes, 1930, Atlantic salmon; etc.).

[5] Unpublished data. It is difficult to obtain precise records because the young are frequently eaten by the parent and are so small as to be easily missed. Rather constant optimal conditions and mature females in good health are required to demonstrate this. Small females will not show it clearly. On the whole it is remarkable that this tendency toward even spacing of the broods is as frequent and demonstrable as the data suggest it to be.

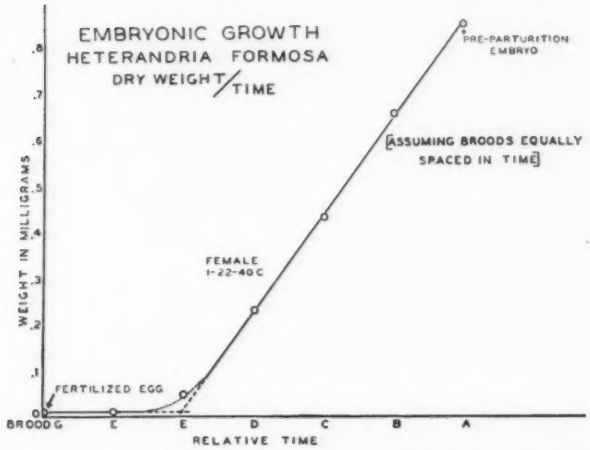

FIGURE 9. The average dry weight of the embryos in each brood of a female with eight fertilized broods is plotted as a function of time. Since the actual age of the embryos cannot be determined, no specific time units can be employed. However, in any large, mature female of Heterandria kept under reasonably constant conditions the broods of embryos seem to be born at approximately equal intervals. Therefore, the broods of embryos still in the ovary can be equally spaced on a time axis without the necessity of specifying the actual number of days or hours between them. Since the time relations between the various broods contained in the ovary are thus represented without using known time units, a relative time scale is actually employed. This concept of relative time is also used in Figures 10–12 and throughout the text. The female has been kept under constant illumination in a room with only slight variations in temperature.

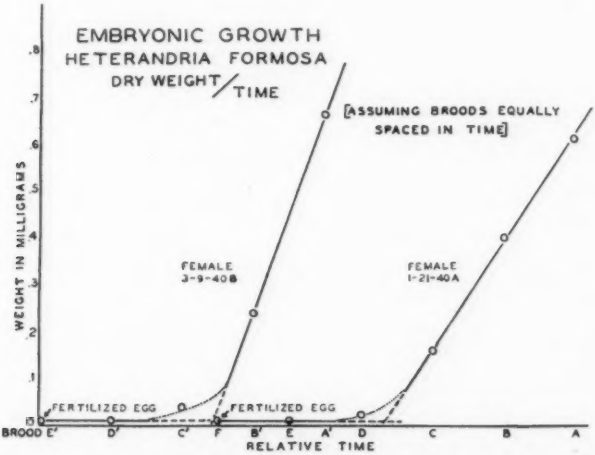

FIGURE 10. The average dry weight of the embryos in each brood of two different females, one with five and the other with nine broods, is plotted as a function of relative time. The treatment is the same as in Figure 9.

could be estimated by comparison of the ovisac diameters with the average weight of the embryos as shown in Figure 7. The results were plotted exactly as in the above cases where the weights were obtained directly. Despite the wide variety of ages and conditions represented, the majority showed the generally linear nature of growth in the two periods. Data from three such females are presented in Figure 11.

This even spacing can also be demonstrated by the superposition of growth curves from different females. Numerous females were examined by this method and their intermediate broods were found to fall on the growth curves of other females. For example, Figure 9 shows the growth curve for the embryos of a female with eight broods. Broods B and E of this female (second and fifth broods respectively) were found to correspond in weight to the first and third broods of

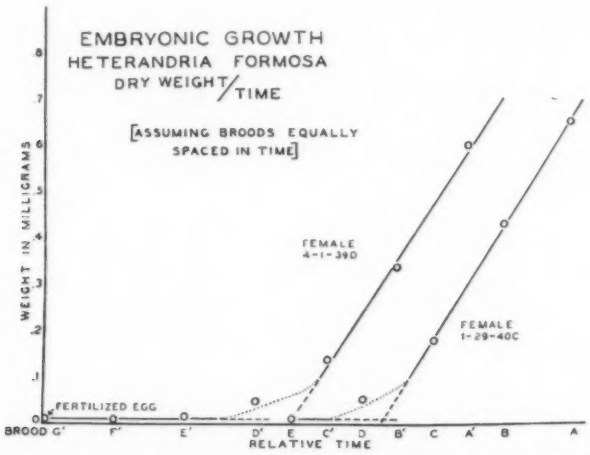

FIGURE 11. The average dry weight of the embryos in each brood of two different females, one with seven and the other with five broods, is plotted as a function of relative time. The dry weight values have been obtained by use of the ovisac diameter and the dry weight curve of Figure 7. The broods are assumed to be evenly spaced in time.

a four-brooded female. When the second brood of the four-brooded female was arbitrarily placed midway between broods B and E on the time axis, it was found to have a weight value which placed it on the growth curve representing the embryos of the eight-brooded fish.

The diameter of the ovisac for each brood of an eight-brooded female can itself be plotted as a function of relative time (Fig. 12). The resulting curve is smooth and sigmoid, showing a slower diameter increase during the early and late periods. In the female represented in Figure 12 the earliest brood was observed to represent recently fertilized ova, and the latest brood was found to represent embryos ready for parturition. Hence a point midway on the relative time axis should indicate the ovisac diameter of an embryo roughly half way through development. It is possible in this fashion to determine the percentage of development represented by other ovisac diameters.

In the course of these studies on Heterandria the specific stage of development corresponding to various ovisac diameters has been noted. It is now possible to assign the percentages of development determined for certain ovisac diameters to developmental stages in Heterandria. These can then be compared with similar stages described in closely related fish in which a more direct measure of time can be obtained. Bailey (1933) faced with similar difficulties in determining the exact time of development in the poeciliid *Xiphophorus helleri,* staged 50 ova and embryos and selected the tenth, twentieth, thirtieth, etc. as representing corresponding percentages of development. *Fundulus heteroclitus* was studied at the Marine Biological Laboratory, Woods Hole, in July 1939 and together with Xiphophorus was compared with Heterandria. The relative times between common stages were

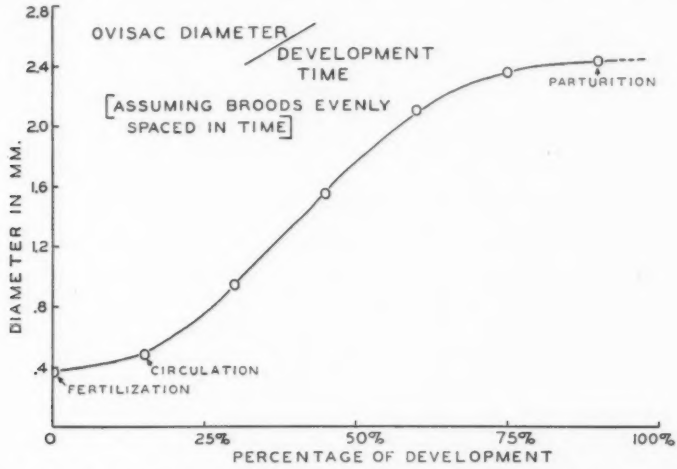

FIGURE 12. The average ovisac diameter of each brood in a female with eight broods is plotted as a function of percentage of development. This is the same female shown in Figure 6 and the time units are obtained in the same manner. However, since the entire range from the fertilized egg to the embryo ready for parturition is represented, these extremes are indicated as 0 per cent and 100 per cent of development and the relative time axis is subdivided accordingly.

clearly similar in the three species. Although the comparison could be only approximate,[6] it reduced the likelihood of a serious error in the time relations assigned to Heterandria.

If these time relationships are used to determine the increments of growth per unit of time during the later embryonic period, it will be seen that the increments remain constant. When the percentage of maximum increment of dry weight is plotted as function of time in Heterandria (Fig. 13), the result is a straight line parallel to the time axis throughout most of the embryonic period. Gray, treating

[6] In addition to the difficulties of comparing stages described for different species, the relative time between embryonic stages may be changed by exposure to different temperatures. At the same temperature the relative time between similar stages in two different species may be different (Moore, 1939; Worley, 1933).

his growth rate data for the trout in this manner, demonstrated an asymmetrical rise and fall (Fig. 13). This represented a deviation from Robertson's formula (1923) in which the percentage of maximum increment plotted as a function of the size of the embryo shows a symmetrical rise and fall. This difference Gray was

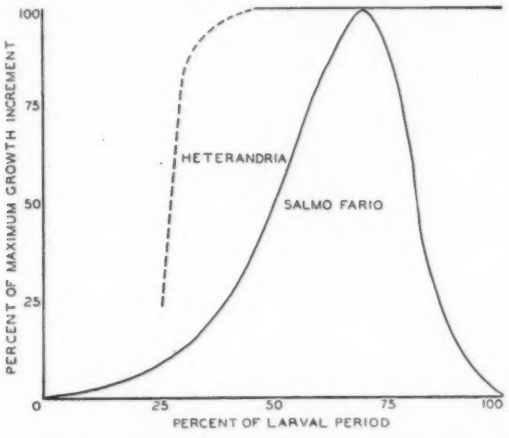

FIGURE 13. The percentage of maximum increment of embryonic growth is plotted as a function of the per cent of the larval period for both *Heterandria formosa* and Salmo fario. No measurements are available for the early stages of Heterandria and the growth increments are necessarily inferred. The curve for Salmo also represents the product of the dry weight of the embryo times the dry weight of the remaining yolk plotted as a function of the per cent of larval development.

able to explain by demonstrating that the growth rate is also a function of the yolk remaining in the yolk sac.

DISCUSSION

The fact that *Heterandria formosa* represents the development of a true viviparity in which nearly all of the nourishment for embryonic development comes from the mother is of interest in itself. When the assumption of equal spacing of broods is made and the data treated accordingly, it also appears that the nourishment for the growth process is being used by the embryo at a constant rate. If this is true, it suggests that some specific extrinsic factor or factors is limiting embryonic growth in this species. Restricted food supply and limited oxygen availability were considered likely to affect the growth rate in this manner. The discussion which follows is an attempt to evaluate these two factors.

It has been shown that morphogenesis in the oviparous fish adjusts itself to the amount of nourishment available, i.e. that the size of the larva is dependent on the amount of yolk available (Morgan, 1896) and not on the total amount of cytoplasm (Sumner, 1900; Hoadley, 1928). That the rate of growth of the oviparous fish embryo is dependent not only on the mass of the embryo but also on the actual amount of yolk remaining has been shown by Gray (1926; 1928a, b) (cf. Fig. 12).

It is conceivable that the pseudo-placental barrier itself may increase in effectiveness only enough to allow for the steadily increasing demands of the maintenance metabolism. Such a relationship seems rather remarkable under the circumstances and might be expected to break down with the complications of retraction of the pericardial sac and development of special secretory cells in the follicle wall. No alteration in growth rate can be detected when these changes in the pseudo-placental barrier occur.

Instead of the limitation lying in this barrier, it may be that certain of the raw materials for growth are present in the maternal blood stream in limited amounts. How such a limitation could affect all of the broods in a similar manner is not clear. There is, however, some indirect evidence that the growth of the individual embryo is responsive to changes in the total maternal supply of nourishment available to all the embryos.

This evidence involves the young of fish not heavily burdened with embryos. Since the straight line nature of the growth curve cannot be tested unless more than four broods are present, all of the data used to develop the idea of a constant increment of growth have been obtained from females with many broods. In these the food requirements of the embryo must constitute a great drain on the mother. The physiological drain would not be as great in females which are recovering from unfavorable conditions, because they contain fewer broods than they are capable of supporting. In these females more nourishment per embryo should be available than in females which have been kept under relatively constant conditions. If more nourishment is available, the embryos should be larger at the end of the larval period (cf. Gray, 1928a). The actual results in Heterandria are that the first young born of a female recovering from unfavorable conditions are large.[7] This constitutes the best available evidence that the food supply of the embryos in a many brooded female is restricted in some manner, and supports the hypothesis that during the main growth period food may be the determining factor in the development of Heterandria at normal temperatures.

There is considerable evidence that at higher temperatures the oxygen supply to the embryo may be a factor limiting growth. According to Gray, all of the oxygen used by the trout embryo is for maintenance metabolism, the amount used for growth being almost negligible. He also found that a large drop in the growth rate of the trout caused no corresponding drop in oxygen consumption. Nevertheless, Jacques Loeb showed (1894) in Fundulus that development is directly retarded by lack of oxygen. The Heterandria females may under certain conditions at 25° temperature give birth to young regularly every four days. A rise in temperature of two or three degrees during the daytime for even two or three days may delay the next brood.[8] It seems likely that the decreased oxygen supply may account for

[7] The following figures will not be discussed in detail but are presented in support of the statements made above. A female recovering from unfavorable conditions was observed to give birth to young on Dec. 13 and 14 after a lapse of several months. These young were very much larger than those born four and ten days later. The actual dry weights were found to be:

Dec. 13 and 14, 1940	Dec. 18 and 26, 1940
.88 mg.	.63 mg.
.95 mg.	.64 mg.
.89 mg.	.61 mg.

[8] Scrimshaw—unpublished data.

this. At the higher temperatures both the mother and the embryos require more oxygen per unit time, but less oxygen is dissolved in the water. It also seems likely (cf. Irving, 1941) that the oxygen dissociation curve of the hemoglobin in the blood would be shifted to the right and flattened, and as a result the oxygen carrying power of the blood would be reduced by the increased temperature.

It can also be observed that a female kept at a constant temperature of 25° C. will have a number of its embryos dying within several hours when the temperature is raised to 28° C.[9] Such a temperature is not in itself supra-maximal, for the temperature tolerance of the mother runs well above 34° C. The young after birth grow well at this high temperature. The embryos at all stages of development seem to tolerate this temperature satisfactorily when isolated from the mother if the water is well aerated. For example, their heart rate shows no irregularity in μ value on an Arrhenius plot until the temperature reaches 34.6° C. The death of the embryos in the above case can be explained on the basis of limited oxygen supply to them.

Oxygen supply is not likely to be the limiting factor at ordinary temperatures. When exposed only to natural daylight, a female kept at a constant temperature and under approximately uniform feeding conditions will contain a certain number of embryos and these will show the constant growth increment described. When such a female, other conditions remaining the same, is exposed to continuous artificial light for about a month, the number of embryos markedly increases.[10] There is no reason to believe that the total availability of oxygen has significantly increased.

It seems reasonable to believe that the oxygen is the principal limiting factor at higher temperatures, and food supply at the moderate ones. This would mean an intermediate range in which the two factors are complementary in a regular fashion. The data do not serve to distinguish between different degrees of limitation. Furthermore, Heterandria kept at temperatures high enough to limit the oxygen supply do not have enough broods to enable the growth rate to be determined. The possibility of other factors such as endocrine balance influencing the growth rate has not been excluded.

Summary

The fertilized egg of the viviparous poeciliid *Heterandria formosa* is minute and is made up almost entirely of a single large oil globule. At least 70 per cent of the original volume of the oil globule is still present when maternal contribution of nourishment begins. Secretory cells develop in the ovisac wall late in the embryonic period. These together with adaptations previously described permit the mother to contribute nearly all of the raw materials for growth and development of the embryo after the egg has been fertilized.

The increase in wet and dry weight of the embryos at the expense of the mother has been determined by obtaining both wet and dry weights at various stages of development. The dry weight of the embryo increases from 0.017 milligrams at the time of fertilization to 6.8 milligrams at the time of parturition. The percent-

[9] Scrimshaw—unpublished data.
[10] Scrimshaw—unpublished data.

age dry weight remains constant at 13.2 per cent after the pseudo-placental association is established.

Observations of living females suggested that under constant optimal conditions the broods of a single female tend to be evenly spaced in time. Upon this assumption the dry weights of the embryos in each brood of suitable females were plotted against relative age. The resulting curves suggested that the rate of growth after the maternal contribution of nourishment can be detected is approximately constant. Food supply and oxygen supply are discussed as factors which might limit this growth. Embryonic growth in Heterandria is compared with that in oviparous fishes.

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THE CAPILLARY BED OF THE CENTRAL NERVOUS SYSTEM OF CERTAIN INVERTEBRATES

ERNST SCHARRER

(From the Department of Anatomy, Western Reserve University School of Medicine, Cleveland, and the Marine Biological Laboratory, Woods Hole) [1]

The vascular pattern of the vertebrate brain may be either one of two types: the one consists of single vessels that anastomose to form a continuous capillary network; the other consists of paired vessels that end in capillary loops. These two types are, as a rule, mutually exclusive, except in the lungfish, Epiceratodus (Craigie, 1943), and in the salamander, Ambystoma (Craigie, 1938a), where both networks and loops occur. The network pattern is the more common type. It is found in monotremes (Sunderland, 1941) and in all placental mammals, in reptiles with the exception of the lizards, in anuran amphibians, and in the fishes including the hagfish, Myxine. The paired vessels ending in capillary loops are characteristic of the marsupials (Wislocki and Campell, 1937; Craigie, 1938b; Sunderland, 1941), the lizards (Schöbl, 1878; Sterzi, 1904) including Sphenodon (Craigie, 1941a), and the tailed amphibians (Schöbl, 1882; Sterzi, 1904; Craigie, 1938a; 1939; 1940a) including the Gymnophiona (Craigie, 1940b; 1941b). The brain of the lamprey, Petromyzon, is also supplied by loops (Craigie, 1938a).

The study of patterns of cerebral vascularization has been extended here to include invertebrates. In most invertebrates blood vessels do not enter the nervous tissue. There are, however, exceptions. Havet (1916), for instance, in his investigation of the glia cells of the invertebrates mentions the existence of blood vessels within the central nervous system of the earthworm. Another reference may be found in Cajal's (1929) paper on the origin of unipolar neurons in invertebrates according to which the cerebral ganglia of the squid are vascularized by intraganglionic blood vessels.[2] Both of these animals, the earthworm and the squid, have been studied, therefore, and the blood vessels supplying their ganglia have been compared with those of vertebrates.

Material and Methods

Large earthworms (*Lumbricus terrestris* [3]) were collected on lawns in the Cleveland area during rainy nights and were fixed with Zenker-formol. The ring consisting of cerebral and subesophageal ganglia and their connectives was embedded in paraffin and cut 7 micra thick. The sections were stained with Masson's

[1] This research was aided by a grant made to Western Reserve University by the Rockefeller Foundation.

[2] The papers of Williams (1902) and Grimpe (1913) give excellent accounts of the vascular system of cephalopodes, but do not include descriptions of the vascularization of the cerebral ganglia.

[3] *Lumbricus terrestris* was introduced from Europe and has become widely distributed in Ohio in the past 25 years (Eaton, 1942).

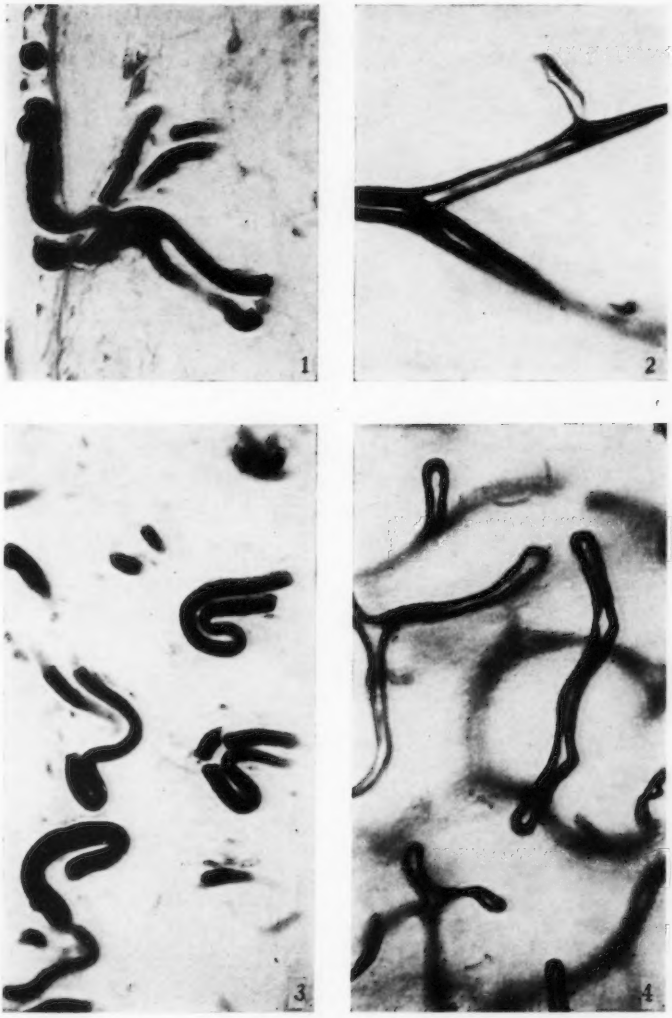

FIGURE 1. A pair of branching blood vessels in the central nervous system of the earthworm. Zenker-formol, paraffin, 7 micra, Masson's trichrome stain. Photomicrograph, × 350.

FIGURE 2. A pair of blood vessels in the brain of the opossum branching in the same manner as those of the earthworm shown in Figure 1. Injection with India ink-gelatin, formalin, nitrocellulose, 100 micra. Photomicrograph, × 350.

FIGURE 3. Terminal loops in the central nervous system of the earthworm. Technique and magnification as in Figure 1.

FIGURE 4. Terminal loops in the brain of the opossum. Technique and magnification as in Figure 2.

trichrome stain. Probably because of the strong contraction of the animals when the fixing fluid is injected into the body cavity, the central ganglia sometimes become very hyperemic. In such animals the blood vessels of the central nervous system are filled with the blood fluid which stains well with the red component of the Masson stain (see Figs. 1 and 3).

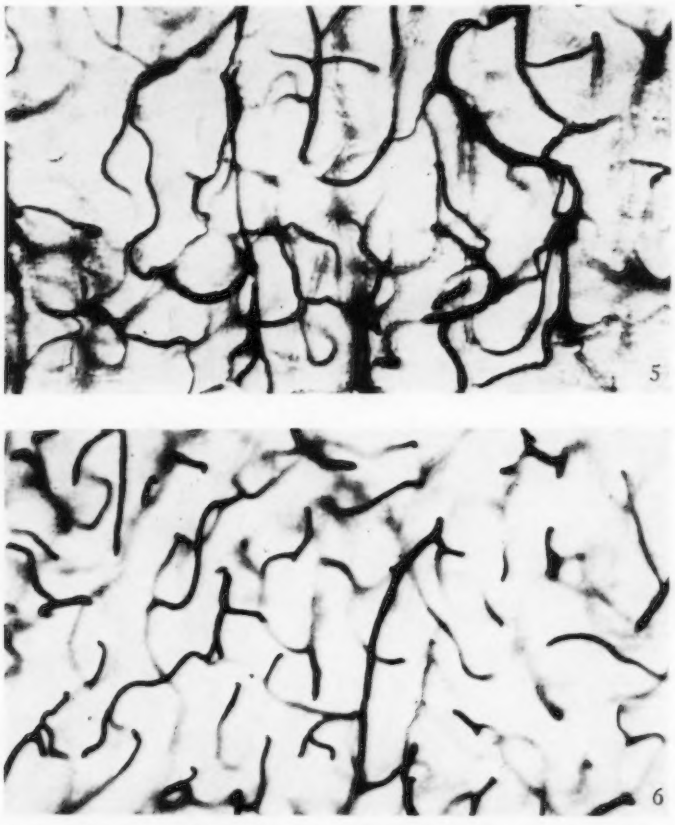

FIGURE 5. Capillary network in the cerebral ganglion of the squid. Injection with India ink-gelatin, formalin, nitrocellulose, 100 micra. Photomicrograph, × 220.

FIGURE 6. Capillary network in the brain of the rat. Technique and magnification as in Figure 5.

Squids (*Logligo pealii*) were obtained at the Marine Biological Laboratory at Woods Hole and were injected with India ink-gelatin through the heart in the same manner as vertebrates. The injected cerebral ganglia were fixed in formalin, embedded in nitrocellulose, and sectioned 100 micra thick. The blood vessels observed in the central nervous system of both the earthworm and the squid were

compared with those in various vertebrates, including monkey, cat, guinea pig, rat, opossum, alligator, and several species of teleosts. These were all injected with carmin- or India ink-gelatin, were embedded in nitrocellulose, and were sectioned 100 or 200 micra thick.

Observations

A comparison of the illustrations (Figs. 1 to 4) shows that the blood vessels of the central nervous system of the earthworm are of the same type as those of the opossum brain. In the earthworm blood vessels enter the tissue of the central nervous system in pairs. They divide together and their branches form corresponding pairs (Fig. 1). Finally the two limbs of each pair join and thus end in hairpin-like loops (Fig. 3). This is essentially the same arrangement which Wislocki and Campbell (1937) described in the opossum where arteries and veins stay together in pairs after they have entered the brain tissue. Whenever an artery divides, the accompanying vein divides the same way (Fig. 2), and all blood vessels within the opossum brain end finally in non-anastomosing loops (Fig. 4).

The vascular pattern of the central nervous system of the squid is entirely different from that of the earthworm. In the squid arteries and veins enter the cerebral ganglia singly. Their branches form a network of anastomosing capillaries (Fig. 5), just as in the brain of placental mammals (Fig. 6).

Discussion

"Since the discovery by Schöbl (1878) that there exist in reptiles two radically different types of cerebral vascular bed, one reticular and the other composed of independent, non-anastomosing capillary loops, the relationship between these two types has remained obscure and attempts to reconstruct the phylogenetic history of this mechanism have been complicated rather than simplified by increasing knowledge of the occurrence of the loop arrangement in various vertebrate classes" (Craigie, 1941a, p. 263). The difficulties inherent in the application of the phylogenetic concept to the cerebral vascular patterns are well illustrated by the fact that among the cyclostomes, a group of primitive vertebrates, one (Petromyzon) shows loops, another (Myxine) a network (Craigie, 1938a). The description presented here of loops in the earthworm and of a network in the squid only serves to accentuate these difficulties.

An attempt is made here, therefore, to illustrate a common origin of both systems by dispensing with the phylogenetic aspect altogether. The loop system is not considered as a primitive forerunner of the network pattern, but is presented as the result of a parallel development capable of differentiation and functional efficiency corresponding to that of the reticular type.

The origin of the cerebral vascular system may be compared with that of the endocellular blood vessels in the large extramedullary nerve cells of certain fishes such as the swellfish, *Spheroides maculatus*. In young specimens each one of these cells is surrounded by a network of blood vessels. As the cells become larger in older animals the distance between the center of the cell and the blood vessels apparently becomes too great and the blood vessels enter the cytoplasm. Similarly the whole nervous system while still small could be vascularized by a network of superficial blood vessels (Fig. 7 AB). Such a condition actually obtains in the

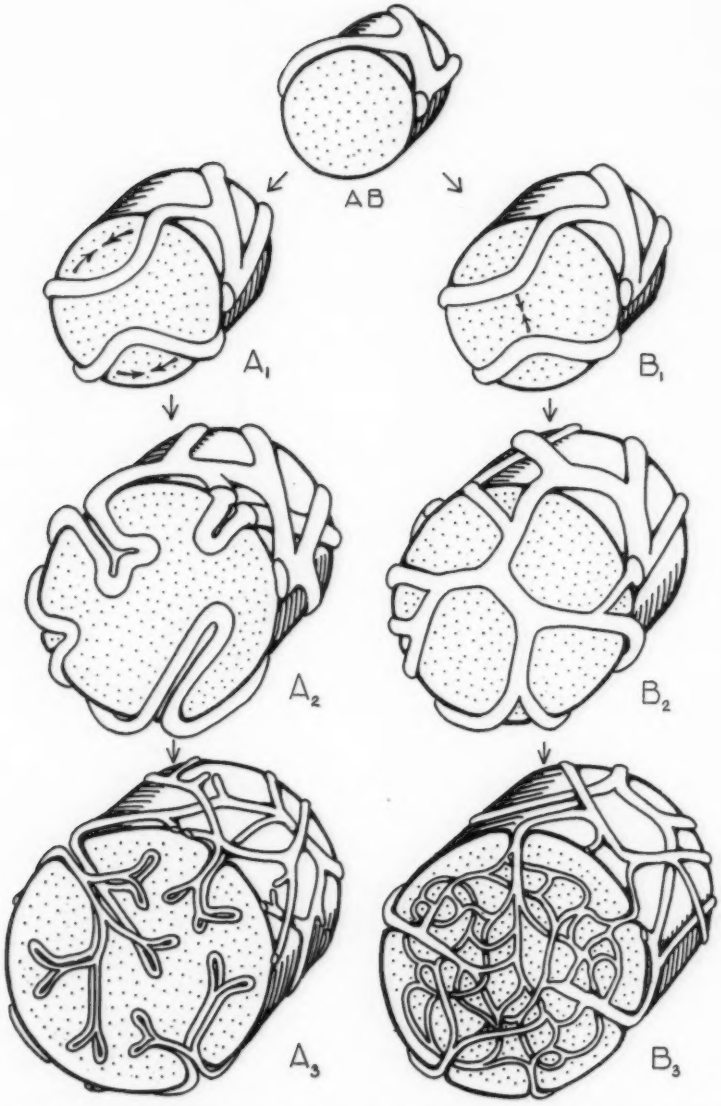

FIGURE 7. Diagram illustrating the derivation of loops and network patterns from a common origin. AB, primitive central nervous system vascularized by superficial network. A_1 and B_1, vessels of the superficial network come to lie within the nervous tissue. There are two possibilities of further development: the blood vessels approach each other in the direction of the arrows (A_1) and thus become paired, or they send out branches which anastomose (B_1). In the one case loops are formed (A_2), in the other a network results (B_2). Both these types are capable of further development to more complex systems (A_3 and B_3).

central nervous system of Amphioxus and in the spinal cord of Petromyzon which are vascularized by networks of superficial blood vessels. With the increase in size of the central nervous system segments of vessels forming part of the surface network come to lie within the nervous tissue (Figs. 7 A_1 and B_1). From this stage both the paired vessels ending in loops (Fig. 7 A_2) and the network (Fig. 7 B_2), may be derived as indicated. Both types occur in invertebrates and vertebrates, and both become eventually highly complex in mammals (Figs. 7 A_3 and B_3).

In this scheme the position of the animal in the phylogenetic order is not considered. This means that the step from A_1 to A_2 or from B_1 to B_2 can be taken anywhere within the vertebrates or the invertebrates. Thus the earthworm follows A_1 to A_2, the squid B_1 to B_2. Among the cyclostomes Petromyzon follows A_1 to A_2, Myxine B_1 to B_2. Epiceratodus and Ambystoma combine the two patterns, a situation which is not illustrated in Figure 7, but which can easily be visualized.

The question still remains: Which factors cause the cerebral blood vessels of the earthworm, of Petromyzon, of the opossum, etc. to differentiate as loops, and those of the squid, of Myxine, and of most vertebrates as a network. An answer to this question is to be expected from the study of the early development of cerebral blood vessels and from the application of the methods of experimental embryology.

Summary

The blood vessels supplying the central nervous system of the earthworm are of the same type as those in the brains of tailed amphibians, lizards, and marsupials, i.e. the blood vessels are paired and end in loops. The blood vessels in the cerebral ganglia of the squid form a network like that which occurs in the brains of fishes, anuran amphibians, reptiles (except lizards), birds, and placental mammals. The origin of both systems is discussed.

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BIOLOGY OF THE CALIFORNIA SEA-MUSSEL (MYTILUS CALIFORNIANUS). III. ENVIRONMENTAL CONDITIONS AND RATE OF GROWTH[1]

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In the cultivation of oysters, clams and other mollusks, as well as in studies on their rates of growth under natural conditions, it has been frequently observed that certain years are more favorable than others for rapid increment in size. But there has been no satisfactory analysis of the environmental conditions responsible for the observed differences in growth rates. For this reason an experimental study, extending over four years, has been made of the growth of the California sea-mussel at the pier of the Scripps Institution of Oceanography. At this station daily records are made of the temperature of the water and of the numbers of dinoflagellates, diatoms and bacteria present and potentially contributory to the mussels' ultimate food supply. For the temperature records the writers are indebted to Capt. S. W. Chambers; for the data on phytoplankton to Prof. W. E. Allen, and for those on bacteria to Prof. C. E. ZoBell. They also appreciate the technical assistance of Miss Harriet Dunn and Mr. Carl Johnson.

In two previous papers (Coe and Fox, 1942; Fox and Coe, 1943) the writers have presented evidence relative to the normal rates of growth in this species at different seasons and at different ages and sizes, the different rates in the two sexes, the nature of the food materials and the influence of environmental conditions on the rates of growth. It was concluded from these observations that there is a generally positive correlation of the growth rates both with temperature changes and with the abundance of dinoflagellates present in the water. No similar correlation was found relative to the numbers of diatoms or bacteria. It was emphasized however that the correspondence between the size of the dinoflagellate populations and the growth rates of the mussels was not to be interpreted as the direct effect of dinoflagellates as potential food material, since a large proportion of the living dinoflagellates ingested usually pass apparently unchanged through the mussel's digestive tract. Furthermore the total supply of living phytoplankton which the mussel could possibly obtain is estimated to be so small in amount that even if all the constituents could be fully utilized they would furnish less than one-fifth of the mussels' nutritive requirements. The principal portion of the food was shown to consist of finely divided organic detritus, derived from the disintegration of many kinds of marine animals and plants, including both unicellular and multicellular forms (Fox and Coe, 1943).

Continuation of those observations during three additional years has shown that there are wide variations in the mussels' growth rates, not only from month to

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month but also from year to year. It is the object of this paper to record these variations and particularly to present such evidence as has now been obtained as to their causes. For this purpose the changes in the environmental conditions from month to month have been analyzed as fully as possible. Since there are no rivers in the vicinity and the annual rainfall is small, there is but little variation in salinity. The principal effective variables are the temperature and the food supply.

The experimental mussels were kept in wire-screened boxes immersed in the sea below the low-tide level. Since the increment in size was found to vary with the age of the individual under identical environmental conditions (Coe and Fox, 1942), it was necessary to have the same ages represented at all times. This required the addition of young individuals from month to month and the removal of the oldest.

The experiment was continued from January 1940, to January 1944, with the exception of the first five months of 1942. At nearly all times the experimental boxes contained from 100 to 400 or more individuals, each age group being in a separate compartment. The average age remained nearly constant and all were sexually immature. When the individuals of a group were separately numbered, it was found that some grew rapidly for a period and were then overtaken by others; some became leaders for several months, while others remained dwarfs. For statistical purposes it was therefore desirable to follow the growth of 20 or more individuals of each age group. The mean monthly increments in size for all groups are shown in Figures 1 to 4.

In any consideration of the environmental conditions, it must be kept in mind that these conditions are constantly changing, due to the water currents that continually sweep past the pier at rates averaging from four to five miles per day. Consequently these conditions may vary considerably from day to day and even from hour to hour. The monthly means, however, will give a reasonably close approximation to the prevailing environments.

A comparison of the graphs in Figures 1 to 4 shows that the growth rates of the mussels have varied considerably, not only from year to year but also for the corresponding months of the years. The mean monthly increment in length for all groups was 3.43 mm. in the year 1940, 3.96 mm. in 1941, 5.43 mm. during the last seven months of 1942 and 5.11 mm. in 1943. It is evident from these figures that 1942 and 1943 were more favorable for rapid growth than either of the other two years and that 1940 was the least favorable. The lowest rate for any month of the four years was in August 1940. During that month the mean increment in length was only 1.6 mm., which was less than one-third as great as in the corresponding month of each of the three other years. The maximum rate occurred during April in 1940, during June in 1941, during July in 1942 and during May and July in 1943 (Figs. 1–4).

An examination of the environmental conditions, particularly as concerns temperature, storms and abundance of the phytoplankton during these years, will give some indication of the influence of each on the observed growth rates of the mussels.

First Year, 1940

The monthly growth rates of the mussels during this year were exceptional in that they showed fewer positive correlations with the temperature and with the

abundance of dinoflagellates than in any of the other years. Following a decrease in the rate during February there was a rapid increase to a maximum in May, fol-

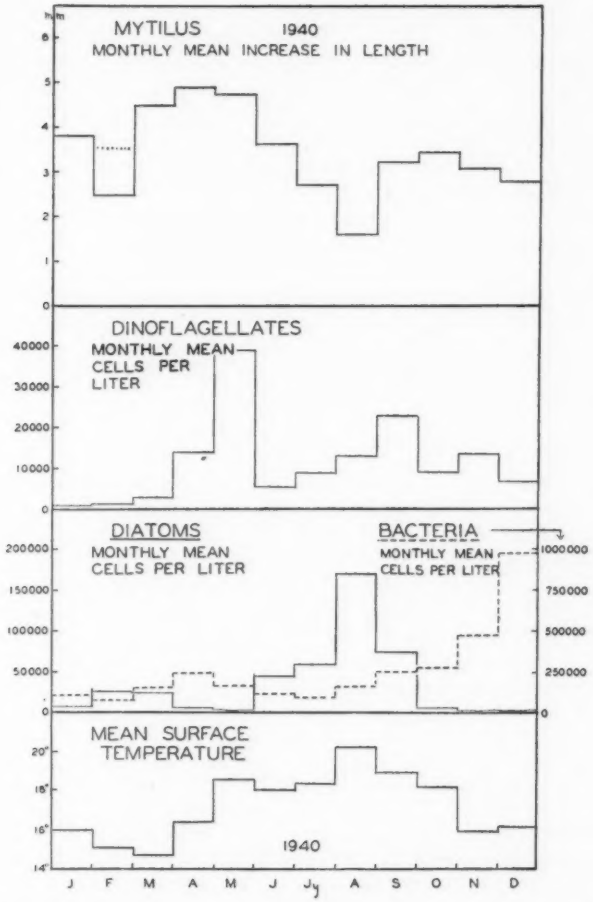

FIGURE 1. Graphs showing the average monthly growth rate of 453 mussels, divided into 11 groups according to age, and the abundance of dinoflagellates, diatoms and bacteria, as well as the average monthly temperature of the water during the year 1940. The depression of the growth rate in February was mainly due to a reduction of the feeding period to 22 days because of accidents caused by storms; the dotted line indicates the estimated increment if the accidents had not occurred. It was necessary also to estimate the growth in December because of an accident due to storm. The numbers of dinoflagellates shown in the graph differ in several cases from those indicated in Figure 4 of our previous paper (1942) because of erroneous data supplied to us at that time.

With some exceptions the growth rates were highest during those months having large dinoflagellate populations and in which the temperature exceeded 16° C.

lowed by a continuous decline to the lowest rate for any month of the four years in August (Fig. 1). The only explanation that can now be given for this exceptionally low rate in August is that for some unknown reason the organic detritus which furnishes the greater part of the mussel's nutrition was not present in sufficient quantity. The sharp drop in February was in part due to storms which necessitated removing the mussels from the sea and keeping them in the aquarium for six days. Since no increase in size occurs in the aquarium except when additional food is supplied (Coe and Fox, 1942), there was a possible feeding period of only 22 days during that month. Computed on the basis of the growth during that period, the estimated increase per day in February would be but slightly less than during the preceding month, as indicated by the dotted line in the graph (Fig. 1). A sharp rise in the growth rate in September and an additional increase in October was followed by the usual decline during the last two months of the year.

As a general rule, but with some conspicuous exceptions, the most rapid increment in size occurred during those months in which a large population of dinoflagellates was present and in which the temperature exceeded 16° C. Neither the diatoms nor the bacteria showed definite correlations with the growth rates of the mussels (Fig. 1).

Second Year, 1941

During the second year the growth rate was somewhat higher than in the preceding year, although the average number of dinoflagellates was smaller and the diatoms were less than half as abundant as in 1940. With the exception of February there was a continuous rise in the growth rate to a maximum in June, with a steady decrease thereafter (Fig. 2). The dinoflagellate population correspondingly reached a maximum in July, followed by a continuous decline to a minimum in December. Neither the diatoms nor the bacteria showed similar trends. The rate indicated for December is lower than it would have been except for a severe storm which allowed a feeding period of only 27 days.

Third Year, 1942

The experiment was interrupted for the first five months of 1942, but the last seven months of the year showed a greater increment in growth than in the corresponding months of any of the other years. The maximum rate occurred in July, followed by a continuous decrease during the rest of the year, with the exception of a slight rise in November, followed by a small decrease in December (Fig. 3). The water during those months contained an average of more than five times as many dinoflagellates as in the last seven months of the preceding year and the average monthly increment in the lengths of the mussels was 5.4 mm. as compared with 4.3 mm. in the corresponding period of 1941. The average number of diatoms was smaller than in any of the other three years.

Fourth Year, 1943

During the year 1943 the average monthly increment in size was considerably greater than in 1940 or 1941 but somewhat less than in 1942 (Fig. 4). By com-

paring the rate for December 1942 (Fig. 3), with that of January 1943, it will be seen that a sharp drop occurred during the latter month. The cause of this decrease in growth rate may have been due to a severe storm which necessitated transferring the experimental box from the sea to the aquarium, where it remained for four days.

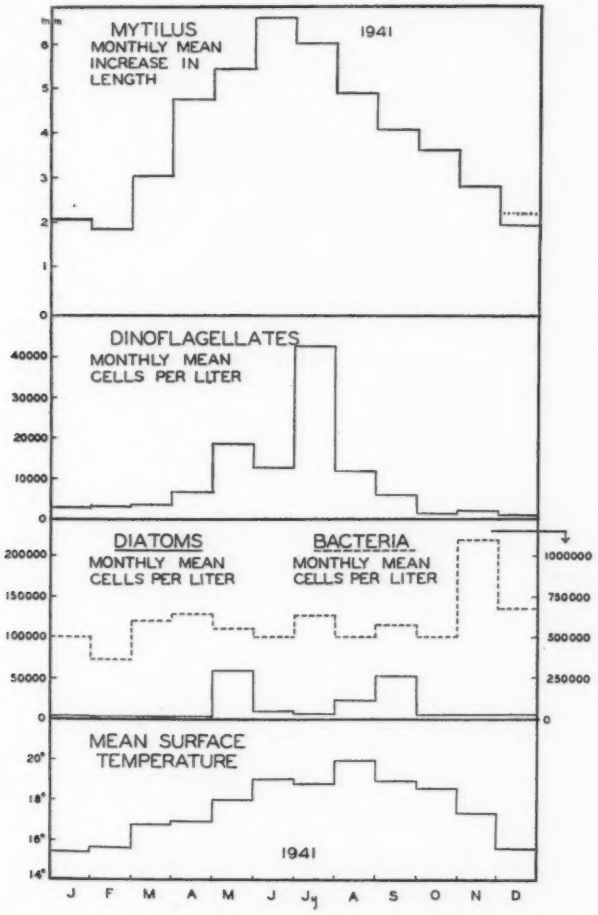

FIGURE 2. Graphs showing average monthly growth rate of mussels and abundance of dinoflagellates, diatoms and bacteria, as well as average monthly temperature of the water during the year 1941. The growth indicated for December represents a feeding period of only 27 days; the dotted line indicates the computed increase for a month of 31 days.

The most rapid increment in size occurred in those months having large populations of dinoflagellates, accompanied, presumably, by an abundance of organic detritus.

From this depression in January, the growth rate increased continuously until May, when the average increment was 6.4 mm. (Fig. 4). An unaccountable drop in the growth rate during June was followed by an average increment of 6.6 mm.

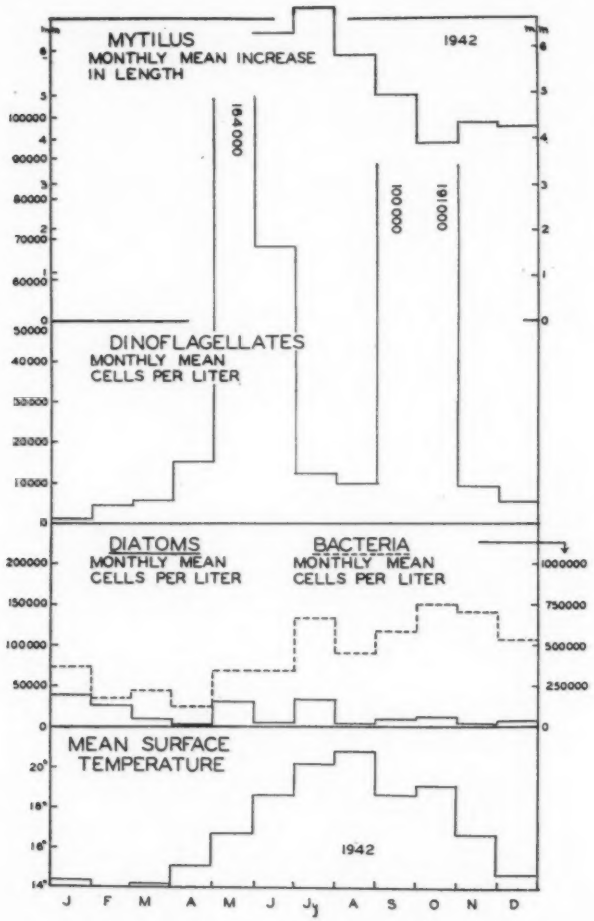

FIGURE 3. Graphs showing the mean monthly growth of mussels, the mean monthly temperature of the water and the mean monthly abundance of dinoflagellates, diatoms and bacteria during 1942.

in July, which was the highest rate for the year. Following the usual decrease in August, the rate continued high and steady during the two succeeding months; then, instead of the usual decline in November, there was a rise to an average of 6 mm., as compared with about 3 mm. in the corresponding month of 1940, 2 mm.

in 1941 and 4.3 mm. in 1942. In December the rate was but little more than half as great as during the preceding month (Fig. 4).

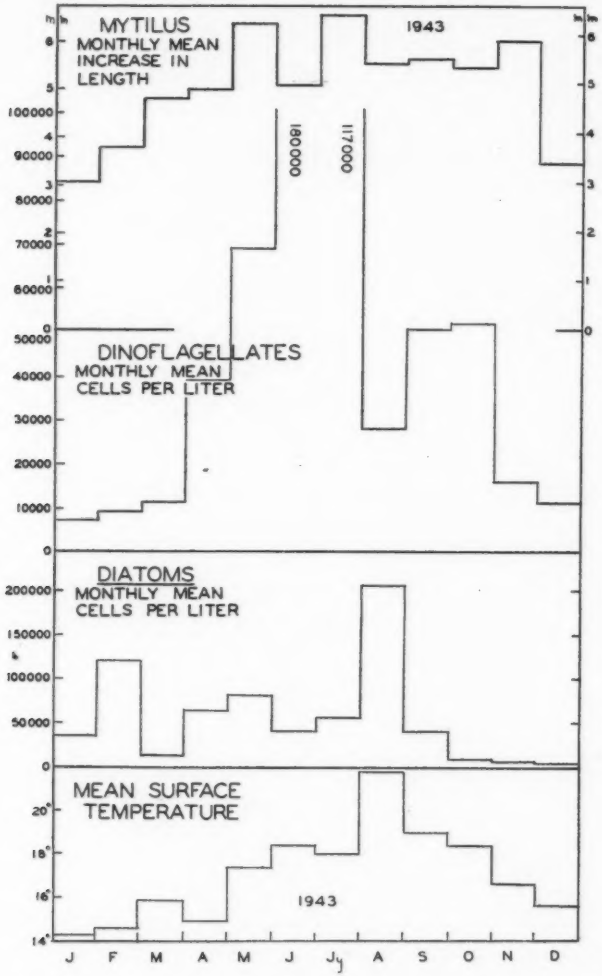

FIGURE 4. Graphs showing the mean monthly growth of mussels, the mean monthly temperature of the water and the mean monthly abundance of dinoflagellates and diatoms during the year 1943.

The average monthly increment in length was 5.1 mm. as compared with 3.43 mm. in 1940 and 3.96 mm. in 1941. This increased rate of growth was accompanied by populations of dinoflagellates more than four times as great as in either

1940 or 1941. The diatoms were also four times as numerous as in 1942, but again it should be emphasized that neither the dinoflagellates nor the diatoms furnish more than a small proportion of the food supply of the mussel.

Environmental Influences

The observations described on the foregoing pages show how great a variation was found in the growth rates of the mussels in different years and in different months of each year. The comparisons of these rates with the temperature and with the abundance of dinoflagellates, diatoms and bacteria are shown in Figures 1 to 4. There are obviously many other environmental conditions which are constantly exerting their influence on the growth of the mussels. Some of these may be of great importance but they are so sporadic in their action or so difficult to measure that no precise evaluation of their influence has as yet been possible.

There is little variation in salinity throughout the year and there is often a correlation between the amount of oxygen and the relative abundance of phytoplankton.

1. *Temperature*

As a general rule the rate of growth in mollusks increases with the temperature to a certain optimum and then rapidly decreases. Consequently the annual increment in length is greater in southern than in more northern localities because of the longer season favorable for rapid growth. The observations of Weymouth, McMillan and Rich (1931) on Siliqua, of Newcombe (1936) on Mya, of Chamberlain (1931) on Lampsilis, of Orton (1926–27) on Cardium and of Coe (1938) on Ostrea support this conclusion. The size eventually reached by the individual however is commonly much greater in the north because of the greater length of life.

At the pier of the Scripps Institution of Oceanography, where the experiment was conducted, the variation in the mean monthly surface temperature of the water during the year seldom exceeds 8° C. Both the low point of about 14° in winter and the high of about 22° in summer are well within range of the normal activities of the mussel. The highest temperature recorded at any time during these four years was 22.9° C., in August 1943, and the lowest was 13.4° in January 1943. Consequently growth continues throughout the year in this locality, although the rate of increment in length is only about half as great in midwinter as it usually is in the early summer. This decreased rate in winter is presumably due both to a lower state of metabolic activity and a decreased supply of nutritive materials.

In *M. californianus*, as in *M. edulis* (Loosanoff, 1942), feeding continues at temperatures both lower and higher than the extremes mentioned in the preceding paragraph. Under experimental conditions the California mussel will secrete byssus threads, ingest food and discharge feces at temperatures as high as 24 to 26° C. and to a less extent at 27 to 28°. The individuals subjected to the highest of these temperatures however died within 5 to 7 days. The lowest temperature at which ingestion and fecal discharge were found to occur was 7 to 8°. Since these mussels were subjected to the temperatures mentioned without any period of acclimatization from an aquarium temperature of 15°, it is considered probable that the figures given do not represent the extreme range of the mussels' potential metabolic activities.

Experiments previously made in this laboratory indicate that the maximum rate of filtration and maximum oxygen consumption take place at about 20° C., with distinctly lower rates below 15°. A decreased growth rate has occurred in August, the month of highest temperature, in each of the four years. But in 1940 the decrease began in June and reached its lowest rate in August, while in 1941 the highest growth rate occurred in June and in 1942 in July. In 1943 maxima occurred in May and July. The variability in the growth rates at corresponding temperatures in different years indicates that the food supply is more influential than small variations in temperature in determining the rates of growth. The prevalence of storms and high seas common in winter are doubtless detrimental to active growth.

2. Food

The mussel is essentially a scavenger, utilizing as food not only small unicellular organisms and dissociated cells but also the particulate disintegration products of any of the animals and plants which die in the vicinity or similar products which are brought from a distance by currents. Even the bacteria which cause the decomposition may themselves be utilized as an additional source of nourishment (Coe and Fox, 1942; Fox and Coe, 1943).

The constituents of the ingested materials have been ascertained at frequent intervals by examination of the stomach and intestinal contents and of the feces. The substances most commonly present are finely divided organic detritus, dinoflagellates, diatoms, silicoflagellates and bacteria; also tintinnids, flagellates, ciliates and other protozoans, as well as algal cells and fragments, algal spores, spermatozoa and ova (including those of its own species), together with inorganic substances such as particles of sand and shells. At times the organic materials may be ingested in amounts much greater than the mussels' capacity for assimilation. The excess, if not too great, may pass unchanged through the digestive system but in case of a very great surplus most of the material is rejected by the palps and is discharged from the mantle cavity as pseudofeces. No satisfactory evidence of selection, except as to size, from among these small cells and particles has been obtained, although chemically injurious substances are rejected, together with the larger cells and other objects.

Digestion in the mussel, with the exception of starch and glycogen, appears to be mainly or wholly intracellular. Many of the smallest objects and particles are phagocytized by the cells lining the digestive diverticula. Others are ingested by phagocytic cells which migrate into the lumens of the stomach and intestine and later return with their ingested materials through the epithelial lining of the digestive tract and thence to the connective tissues of the body, as Yonge (1926, 1931) has so fully described for the oyster. Most of the local dinoflagellates and many of the diatoms are far too large to be assimilated in this manner. Some of the starch and glycogen, on the contrary, undergoes extracellular digestion in the stomach through the action of enzymes in the style. No evidence of the digestion of cellulose, which forms the covering walls of most neritic dinoflagellates, nor of any cells with completely closed cellulose walls, has been obtained (Fox and Coe, 1943).

Diatoms. These organisms, either living or dead, furnish a small portion of

the mussels' nutrition. Their disintegration products are also utilized. They are usually present in numbers ranging from 1000 to 200,000 per liter but the large and spiny ones are not ingested. Many of those that enter the digestive tract are seized and digested by the phagocytic cells mentioned in a preceding paragraph, while others pass through the tract without apparent change.

The mean number of diatoms, as counted by the settling method, per liter of water for each month of the four years is shown in Figures 1 to 4 and the combined monthly averages for 1940, 1941 and 1943 in Figure 5. In none of these years has there been a direct correlation between the diatom populations and the mussels' growth rate, although positive correlations have been reported by Newcombe (1935) for Mya and by Nelson (1942) and others for oysters.

The number of diatoms in the water about the mussel beds has varied greatly from year to year. The average in 1940 was 38,700 per liter, in 1941 16,600, in 1942 12,600 and in 1943 54,300 (Figs. 1–4). The average monthly increase in the lengths of the mussels for the same years was 3.43 mm., 3.96 mm., 5.43 mm. and 5.10 mm., respectively. It is obvious that there was in these four years no direct correlation in the two groups of data. In spite of a four-fold increase in the number of diatoms in 1943 as compared with 1942 there was nevertheless a somewhat lower rate of growth in the mussels. This should not be surprising when it is realized that even if these organisms had been uniformly distributed in the water throughout the year, instead of occurring in dense swarms, the rate of filtration by the mussel is such that an adult animal could have secured no more than 200 million to 800 million per year. If all of these could have been fully utilized they would have furnished only a minute fraction of the material required for the upbuilding of the mussel's tissues and gametes. Such of these organisms as can be ingested by the mussel are so minute that it would require some 600 million to supply one gram of organic matter, while the adult mussel is estimated to need about 40 grams annually (Fox and Coe, 1943).

Bacteria. Bacteria are ingested in vast numbers (Figs. 1–3) but their total mass is so small that they have little quantitative influence on the mussels' nutrition (Fox and Coe, 1943).

Dinoflagellates. It has been mentioned that in each of the four years there was generally, but with some conspicuous exceptions, a rather close correspondence between the monthly and yearly growth rates of the mussels and the abundance or scarcity of dinoflagellates in the water. In 1940 the average daily number of these organisms per liter of water was 12,100, as compared with 9880 in 1941, 54,750 in 1942 and 49,500 in 1943. The corresponding average monthly growth rates of the mussels were 3.43 mm. in 1940, 3.96 mm. in 1941, 5.43 in 1942 and 5.10 in 1943. From these figures alone it may be concluded that the mussels grow most rapidly in those years in which the populations of dinoflagellates are the largest.

More precise evidence as to this association however is furnished by an inspection of the monthly data as shown in Figures 1–4. It has been emphasized in a foregoing paragraph however that a large proportion of the living dinoflagellates, which may be ingested in vast numbers, usually pass apparently unchanged through the intestinal tract and often constitute much of the fecal material. Their cellulose walls cannot be digested by the secretions in the stomach or intestine and there is no satisfactory evidence that they are phagocytized by the cells of the digestive diver-

ticula in any considerable numbers. Following the death of these unicellular organisms however, either before or after entering the mussel's digestive tract, they doubtless form particulate disintegration products which are readily assimilated. It is well known that species of Gonyaulax and less frequently of some other genera may be ingested in such numbers and the contained toxic substance accumulated in such quantity as to cause sickness or even death when the mussels are eaten by man.

It has been shown by Fox and Coe (1943) that the mussel filters the water at such a rate that the available supplies of dinoflagellates, even if they could be fully utilized, would furnish only a small fraction of the food which the mussels require for their growth and reproduction. Assuming a filtration rate of 2.5 liters per hour, or 22,000 liters per year, it would be necessary to have an average population of about 2000 of these cells per liter in order to supply one gram of organic matter in a year. This is only about two and one-half per cent of the amount which an adult mussel is estimated to require annually for the upbuilding of its tissues and gametes. During these four years the water has contained averages of 10,000 to 54,750 of these cells per liter but a large proportion of these were present in such dense swarms that relatively few of those that were drawn into the mantle cavities of the mussels could have been actually ingested; the others were presumably discharged as pseudofeces. Furthermore, as has been stated, many of those that are ingested usually pass through the digestive tract without visible change, while the large and spiny forms are seldom ingested.

Therefore any correlation between the abundance of dinoflagellates and the growth rates of the mussels must be merely indicative of other, associated sources of nutrition. The principal source is organic detritus.

Detritus. The organic detritus ingested by the mussel consists of various fragments of cells or of entire cells of minute size, as well as suspended proteins, lipids and polysaccharides. It may be recalled that the mussel obtains its food by secreting over its gills a thin sheet of mucus to which the particles are adsorbed. The mucus sheet with its attached particles is then drawn into the mouth. There is no evidence that the mussel is capable of securing substances in true solution until these have first been changed to particulate form through the agency of various unicellular organisms (Fox and Coe, 1943).

Consequently it may be concluded that, with the exception of refractory humus materials, cellulose, chitin and other indigestible substances, the total organic constituents of all marine organisms, from the smallest to the largest, are potential sources of nutrient for the mussel. After the death and disintegration of the animal or plant, the residual organic matter, or detritus, may remain suspended in the water for an indefinite period before it chances to enter the digestive system of the mussel. The amount available is obviously subject to great variation locally and it is to this variation that many of the differences in growth rates are ascribed.

Inspection of Figure 5, which indicates the combined average monthly growth rates for three years, will show that the rate increases from a low in January or February to a maximum in May, June and July. This period corresponds with the increasing reproduction of many of the invertebrates in the vicinity and elsewhere along the coast. The striped barnacle (*Balanus tintinnabulum*), for example, has minimum and maximum periods of reproduction coinciding almost precisely with the low and high growth rates of the mussels (Fig. 5). These reproductive peri-

odicities are undoubtedly associated with similar variations in the detritus which the barnacles yield, since a large proportion of the free-swimming larvae die without finding a place of attachment and presumably less than one per cent of those which succeed in transforming to the adult stage survive to reach sexual maturity

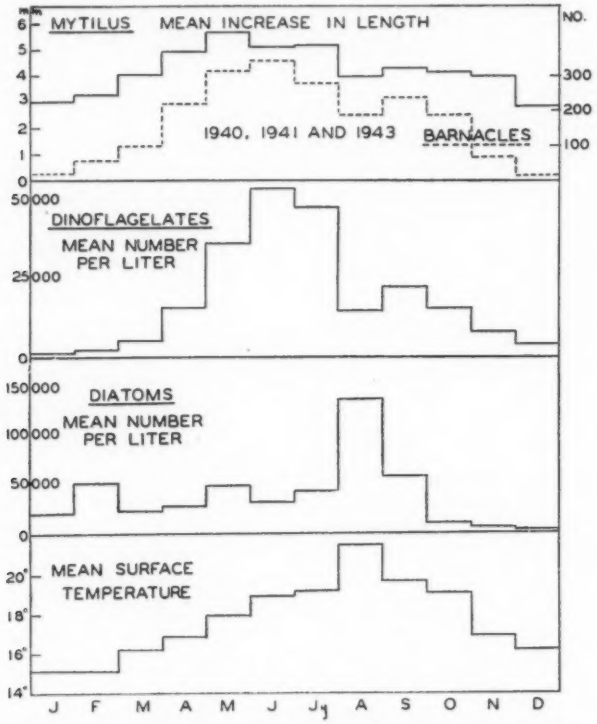

FIGURE 5. Correlations between the mean monthly increment in length of the experimental groups of mussels for the years 1940, 1941 and 1943, the mean numbers of dinoflagellates and diatoms per liter and the mean temperature of the water. The year 1942 is not included because of lack of complete data for the first five months of that year. Except for the decrease both in growth rate of the mussels and numbers of dinoflagellates during August, when the temperatures were highest, the general correspondence of three of these groups of data is evident. But this correspondence does not hold for the diatoms.

As indicative of the relative amount of organic detritus presumably available in each month, the estimated average numbers of barnacles which became attached to each square inch of surface of submerged plates in previous years have been included. These numbers indicate a close correlation with the growth rates of the mussels.

(Coe, 1932). The disintegrated bodies of those that perish doubtless supply more nutritive material to the mussel than can be obtained from the living phytoplankton.

In some years the growth rate has been more or less distinctly bimodal, rising to a maximum in late spring or early summer, followed by an invariable decrease in

August and in three of the four years with another rise preceding the decrease at the end of the year. In three of the four years the dinoflagellates showed somewhat similar bimodal periodicities, with distinct spring and autumn maxima, and this has been shown to be the average condition of these organisms for the twenty preceding years (Allen, 1941). The diatom populations, on the contrary, reached a conspicuous maximum in August in 1940 and in 1943, although in many other years that has been a month of extremely low production.

The bimodal periodicities in the growth rates of the mussel are closely parallel with similar periodicities in the reproduction of many of the associated invertebrates, including the mussels themselves. The growth rates of the entire mussel population would obviously be complicated by the reproductive processes but these complications were avoided in this experiment by using sexually immature individuals.

It is unfortunate that no precise measurements are available relative to the local variations in the amount of organic detritus, but the seasonal differences in the rates of growth of the mussels and other detritus feeders presumably afford a fairly reliable criterion.

Summary

This study offers additional evidence as to the complexity of the environmental conditions found along the shores of the ocean and which affect so profoundly the lives of the organisms residing there. Variations in the growth rates will obviously depend upon the interaction of several of these conditions, not the least important of which are the temperature and the character and abundance of the food supply.

Furthermore these environmental conditions are constantly changing, due in part to the continual motion of the water. At the locality where the foregoing observations were made, there are not only the variable currents caused by wind and tidal changes, but there is also a drift along the coast at a rate averaging four to five miles per day. Consequently the water in which the mussels are living and the conditions associated therewith may differ not only from month to month but also from day to day and even from hour to hour. In one week there may be ten to fifty times as much phytoplankton in the water as in the following week. The yearly averages are more stable but these may vary by more than five fold.

Monthly correlations, extending over four years, between the growth rates of the mussels and the prevalent environmental conditions offer conclusive evidence that the most rapid increase in size takes place at temperatures from 17 to 20° C., although growth continues less rapidly at 14° or lower. Feeding continues at a temperature as low as 7 to 8° and as high as 27 to 28°.

The average number of diatoms per liter was 38,700 in 1940, 16,600 in 1941, 12,600 in 1942 and 54,300 in 1943. The average number of dinoflagellates for the same years was 12,100, 9880, 54,750 and 49,500, respectively. The average monthly increment in the lengths of the mussels was 3.43 mm. in 1940, 3.96 in 1941, 5.43 mm. in 1942 and 5.11 mm. in 1943, indicating a yearly variation of more than 50 per cent.

It is obvious that the two years with the largest dinoflagellate populations have been conducive to the most rapid growth of the mussels, but an increase of four fold in abundance has been associated with an increased growth rate of only 42 per cent. The correlation noted is evidently not direct, since the living dinoflagellates

can supply only a small fraction of the mussels' nutritive requirements. Both mussels and dinoflagellates appear to thrive under the same environmental conditions. That the living diatoms and bacteria are of even less importance in the life of the mussel is indicated not only by the small amount of organic matter that they contain but also by the fact that the mussels grew most rapidly in the year with the smallest number of diatoms and least rapidly in the year when the number was three times as great.

More direct correlations with the growth rates of the mussels are found in the reproductive periodicities of various invertebrate populations which contribute so largely to the available organic detritus and thereby to the mussels' nutrition.

The principal food supply of this species of mussel consists of minute particles of organic detritus derived from the disintegration of the cells of all kinds of marine organisms, both animals and plants, supplemented by living and dead unicellular organisms of minute size as well as living and dead gametes. There is no evidence that organic matter in true solution can be utilized until after it has been changed into particulate form by the action of unicellular organisms.

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PHOTOCHEMICAL SPECTRAL ANALYSIS OF NEURAL TUBE FORMATION [1]

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INTRODUCTION

In recent years the analysis of morphogenesis has been concerned with the study of the chemical nature of substances which play a decisive role in developmental phenomena. In the field of neural induction the usual procedure used is that of extraction of the active material with solvents specific for a particular group of compounds; the degree of substitution is tested by implantation of the extracted material. This approach has failed to yield conclusive results because large quantities of tissue known to contain the normal inductor are not available. Investigators have been unable to determine whether induction resulting from implanted substances is the result of the direct action of the substance on the tissue or of a substance released in the reacting tissue. This technique is also subject to the criticism that two chemical substances may not necessarily be identical because they produce the same histological or morphological changes. It is a well-known fact that histological changes produced in the vagina and uterus by a number of artificial estrogens are identical with those produced by the natural estrogens (McKenzie, 1941).

The investigation of active substances need not be restricted to attempts to isolate them, although isolation and synthesis is the ultimate goal. If the action of a developmental substance is inhibited by a specific agent, a preliminary identification will have been made and it will be certain that the substance inactivated is operative in the organism. The use of chemical poisons has demonstrated the importance of this technique in the field of cellular oxidation. The technique of the photochemical inactivation of substances involved in the developmental processes has enabled the investigator to study the chemical nature of this material during its action in normal development.

The classical experiments of Warburg (1927) in the identification of the respiratory enzyme by absorption spectrophotometry show the importance of the technique of photochemical inactivation. This method involves the irradiation of a biological system with monochromatic radiation and the consequent inactivation of a chemical substance in the system. Absorption is measured indirectly in terms of a physiological or morphological change produced in the biological system. Warburg measured absorption by determining the change in oxygen consumption of yeast cells in the presence of carbon monoxide following irradiation with monochromatic light. In the present investigation absorption is measured in terms of the amount of energy required to inhibit the folding process in neural tube forma-

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tion. By this method an indirect photochemical absorption spectrum of the material involved in a biological activity can be obtained. By comparing this spectrum with the absorption spectra of chemical compounds, information can be ascertained concerning the chemical nature of the material.

A preliminary exploration showed that ultraviolet radiation would not inhibit the transformation of gastrula ectoderm into neural plate unless extremely large doses were used and the cells severely altered or killed. However, the folding process of neural tube formation was inhibited by very weak doses of ultraviolet light with little effect on the embryo in other ways. The neural plate merely continued to develop as a plate. The effect was uniform enough to compare quantitatively the effects of different wave-lengths. Consequently, an attempt was made to identify by its absorption spectrum a substance which is apparently of decisive importance in the process of neural tube formation.

Since most of the work concerned with neural tube formation has been done in Amphibia, it might be expected that this material would offer more advantages than any other. This is not the case. Amphibian embryos possess yolk granules and pigment which absorb and scatter incident radiation. Consequently, the photochemical efficiency curve obtained for inhibition of the folding process in Amphibia would not give a true measure of absorption. For this reason and because an abundant source of avian material was available, it was decided to use chick embryos in this investigation.

The author wishes to thank Dr. Daniel Mazia under whose direction the study was made. Grateful acknowledgment is made to Dr. F. M. Uber who permitted the author to make the thermopile measurements in his laboratory and to Dr. L. J. Stadler for use of the monochromator.

Materials and Methods

The material for this study consisted of the eggs of two breeds of the domestic fowl, the White Leghorn and the New Hampshire Red. In order to secure uniform results, all the eggs were obtained from two pens of hens, one of New Hampshire Reds and the other of White Leghorns. The University of Missouri poultry farm was the source of this material.

Each egg was incubated and the position of the blastoderm determined by candling. The egg was then placed in a Syracuse watch glass which contained modeling clay to hold the egg in place. Only sterile equipment was used in these experiments. The surface of the egg was sterilized with a piece of cotton which previously had been soaked in 70 per cent alcohol. An opening of 7 to 9 sq. mm. was cut in the egg shell by means of a small saw. This revealed the blastoderm through the shell membrane. After removal of the shell membrane with forceps, a sterile .9 per cent salt solution was used to float the embryo to the level of the surface of the egg shell. The age in terms of somites and general condition of each embryo was determined with a dissecting binocular; all embryos in which the neural tube was closed in any region and all abnormal embryos were discarded. The egg shell was marked with a pencil to indicate the position of the embryonic axis so that it could be placed parallel to the slit on the monochromator. The egg, which was tightly fixed in the watch glass by modeling clay, was placed upon a stand which had been attached to a rack and pinion. The egg was elevated until its sur-

face was beneath a quartz prism which was situated at the slit on the monochromator. This "mechanical jack" enabled the investigator to place each embryo the same distance from the source of light. The experimental embryos were irradiated for varying lengths of time with monochromatic ultraviolet light. The control embryos were treated in exactly the same manner except that a glass microscopic slide was placed in the path of the light beam so that no ultraviolet light struck the embryo. Following irradiation, the opening in the egg shell was covered with a piece of a glass cover slip and sealed with a mixture of beeswax and paraffin. The eggs were then incubated for 30 hours or longer; the glass window was always placed down in order to prevent the blastoderm from adhering to the cover slip.

After incubation, the blastoderm was removed from the yolk by cutting around the periphery of it with iridectomy scissors and lifting it off with a metal spatula. The blastoderm was washed in saline and fixed with picro-sulfuric acid. Observations were made upon embryos in alcohol, from whole mounts, and from sectioned material. The whole mounts were stained with borax carmine and the sectioned material was stained with Delafield's hematoxylin or borax carmine.

In the majority of the experiments in this investigation, monochromatic radiation was obtained by means of a large crystal monochromator, described in detail by Uber and Jacobsohn (1938). The monochromator was operated in a horizontal position. Since it was necessary to obtain a vertical beam of light in order to irradiate the embryo, a small quartz prism was placed at the slit on the monochromator. The source of light for the monochromator was a vertical mercury arc which operated at 4 amperes on a 110 volt direct current.

In order to determine the incident dose in ergs/mm.^{2} on the embryos, the monochromatic source was calibrated with a surface-type vacuum thermopile. The thermopile had been calibrated previously with a standard carbon-filament lamp (C–241) obtained from the United States Bureau of Standards. The dosage in ergs/mm.^{2} emitted by the monochromatic source was determined by comparing the deflection which it produced with that produced by the standard lamp.

In the other experiments a mercury discharge tube served as a source of radiation. It was of the Hanovia Sc–2537 type operating at 120 milliamperes and 5000 volts. The transformer was a Jefferson luminous type. Since spectral studies show that such discharge tubes frequently have an additional line around 1800 Å (Landen, 1940), a water filter was placed in the path of the beam in order to absorb the radiation of the shorter wave length. A slit of approximately the same size as that of the monochromator used in this investigation was made on the bottom of the filter in order to approximate the experimental set-up with the monochromator.

Observations

Histological studies of irradiated and control embryos

Chick embryos ranging in age from the primitive streak to the 8-somite stage were irradiated with monochromatic ultraviolet light of wave lengths 2483, 2537, 2576, 2650, 2699, 2804, 2894, 2967, and 3130 Å and subsequently incubated for a period of approximately 30 hours.

The smallest doses produce no detectable changes; the first visible effects to appear as the dose is increased are on the formation of the neural tube. The neural

folds fail to close and instead form flat or half-folded neural plates. In some embryos this occurs in the anterior part of the body; however, in other cases, it is present only in the middle portion. Large doses result in destruction of cells and death of the embryo.

In the consideration of the effect of monochromatic radiation on a developmental process one of the first questions to arise is this: Is it possible to set up a quantitative standard of measurement for comparing the effectiveness of different wave lengths? Such a standard would be a morphological *endpoint*. The procedure would be to compare doses required to attain such an endpoint. If the data are significant, the results should be the same regardless of the particular endpoint chosen. In the present study two morphological endpoints are used: (1) failure of the neural tube to close for a distance of one-third its length in 50 per cent of the cases and (2) failure of the neural tube to close for a distance of one-half its length in 50 per cent of the cases. This investigation is concerned in particular with the embryos irradiated with the amounts of energy necessary to produce these two morphological endpoints. The description is made from a study of embryos in 70 per cent alcohol, sectioned embryos, and whole mounts.

The primary effect of radiation is on the neural plate. Embryos irradiated with wave lengths 2483, 2537, 2576, 2650, 2699, 2804, and 2894 Å are very uniform in appearance. A broad flat plate is present in the anterior one-third to five-sixths of the embryo; the neural tube is nearly always closed in the posterior end. The anterior end of the neural plate bends around the anterior tip of the free head and extends to the ventral surface. The optic cups and infundibulum develop from the portion of the neural plate on the ventral surface of the free head. A lens forms in most cases. A typical case with a broad flat plate, optic cups, lenses, and infundibulum is shown in a section through the anterior end of an embryo irradiated with wave length 2804 Å (Plate I, Figure 1). A section at a more posterior level is shown in Plate I, Figure 2; this embryo was irradiated with wave length 2537 Å. Observations show that the auditory pits are normal in appearance in every case. A group of cells which is probably the neural crest often lies adjacent to the lateral edges of the neural plate. In the region of the rhombencephalon the motor roots of the spinal nerves are present. In the posterior end of the embryo a double neural tube occurs occasionally.

The broad flat plate which is present in embryos of this group is nearly uniform in thickness except in the region of the midline. In this region the plate is thinner (Plate I, Figures 1 and 2). The volume of the broad plate is much larger than that of the neural plate of a 6-somite embryo. Mitoses are abundant on the upper surface of the plate and on the inside portion of the closed region of the neural tube. In a few embryos a small group of cells is present on the surface at the lateral edges of the neural plate. They are filled with granules and irregular in shape. In these cases the lateral ectoderm is similar in appearance to these cells; otherwise, the lateral ectoderm appears normal. In some cases it continues to grow and expand so that a projecting group of cells forms on the dorsal surface of the neural plate.

Another group of embryos is characterized by the presence of a flat or folded neural plate in the middle portion of the body. A complete neural tube forms in the anterior part of the embryo, but it is abnormal in shape and smaller than a

PLATE I

FIGURE 1. Anterior end of an embryo irradiated with approximately 71 ergs/mm.^{2} at wave length 2804 Å and subsequently incubated for 30 hours, showing the broad flat neural plate, optic cups, lens, infundibulum, gut, dorsal aortae, small notochord, and disorganized mesenchyme beneath the neural plate. × 107.

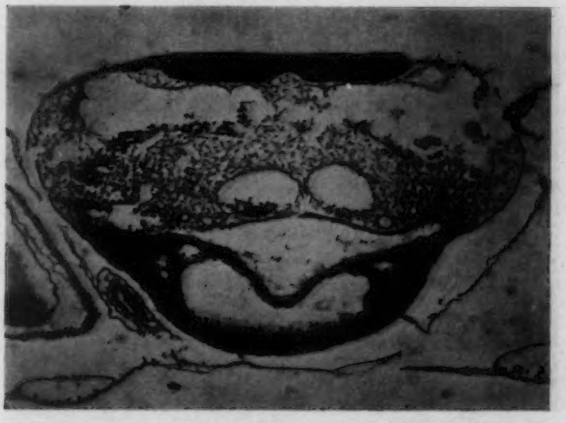

FIGURE 2. At the level of the heart of an embryo irradiated with approximately 95 ergs/mm.^{2} at wave length 2537 Å and subsequently incubated for 30 hours, showing the flat neural plate which possesses a well-defined floor plate, small notochord, dorsal and ventral aortae, heart, and gut. × 107.

normal tube. The typical thin roof plate of the myelencephalon fails to develop. The auditory pits and optic cups are normal.

The cellular appearance of the notochord in the irradiated embryos is the same as that of a normal embryo. Measurements of the cross-section area of the notochord show that it varies greatly at different anterior-posterior levels. The notochord is invariably separated from the neural plate; in the region of the neural tube the notochord lies in contact with the floor plate. In Plate I, Figures 1 and 2 illustrate the small size and relative position of the notochord. As far as can be detected, the somites are normal. In many cases, the mesenchyme beneath the neural plate is abnormally vesiculated in places and considerably disorganized (Plate I, Figure 1). Stained sections show that the mesenchyme beneath the neural plate has been injured; this is suggested by the dark appearance of its cells. The vascular system is well developed; however, the size of the vascular bed is smaller than that of control embryos.

The embryos which were irradiated with wave length 2967 Å are different in appearance from those described previously. The explanation for this is that extremely large doses had to be used in order to produce a detectable effect. In most cases the neural tube forms only in the most anterior part of the embryo. It is very small and abnormally shaped, being extremely flattened dorsoventrally. Occasionally the tube fails to develop and a neural plate is present in the anterior region. In the posterior three-fourths to four-fifths of the body the neural plate is either a disorganized mass of cells or completely absent. The superficial ectoderm appears normal and forms a continuous layer of flat epithelial cells dorsal to the neural plate.

In the embryos irradiated with wave length 2967 Å, the cellular structure of the notochord is normal in appearance. However, the notochord is not in contact with either the neural tube or plate in most regions. The somites are either highly disorganized or absent. The vascular system is poorly developed.

Observations of 111 control embryos which were made from embryos in 70 per cent alcohol, whole mounts, and sections show that they are normal in 100 per cent of the cases. It will be remembered that all embryos were examined immediately before irradiation and the abnormal ones discarded. This explains the fact that all control embryos are normal.

From this description, it is evident that all wave lengths except 2967 Å produce a uniform effect on neural tube formation; consequently, quantitative studies of the relative efficiency of different wave lengths in preventing closure of the neural tube can be made.

The effect of radiation on mitosis and volume of the central nervous system

The purpose of this study is to determine if radiation has a detectable influence on mitosis and volume changes with the low doses used. Mitotic counts and measurements of the area of cross sections of the central nervous system were made on the embryos which were described histologically in the previous section. An analysis of the effect of radiation on mitosis was made by counting the number of mitoses in three sections of the neural plate or tube at each of three different levels. Volume was measured indirectly by determining the cross-section area of one of

TABLE I

Mitotic counts and cross-section measurements of irradiated and control embryos

Group of embryos	Number of mitoses per section per embryo	Cross-section area per embryo	Mitoses per cross-section area unit
	Level *a*		
Embryos irradiated at wave lengths from 2483 to 2804 Å (10)*	45.90±14.7	1189.2±278	.038
Embryos irradiated at wave lengths 2894 and 2967 Å (4)	20.75±9.0	719.5±95	.029
Control embryos (5)	44.80±15.0	1504.6±409	.030
	Level *b*		
Embryos irradiated at wave lengths from 2483 to 2804 Å (10)	7.80±2.6	251.3±34	.031
Embryos irradiated at wave lengths 2894 and 2967 Å (4)	1.75±2.1	93.75±47	.018
Control embryos (5)	12.60±4.7	379±115	.033
	Level *c*		
Embryos irradiated at wave lengths from 2483 to 2804 Å (10)	6.90±2.1	191.5±51	.036
Embryos irradiated at wave lengths 2894 and 2967 Å (4)	4.75±5.6	99.5±85	.048
Control embryos (5)	10.00±2.0	284.60±46	.035

Level *a* consists of three sections adjacent to the anterior end of the notochord; level *b* is represented by the middle section of the central nervous system and this level is determined by counting the total number of sections of the central nervous system; and level *c* is ten sections posterior to the most posterior section in which the neural tube has failed to close in the irradiated embryos and a comparable section in control embryos. In the irradiated embryos in which the counts were made, level *c* is always in the posterior quarter of the nervous system. In determining the number of mitoses per section per embryo, counts were made on three sections at each level of each embryo. The number of mitoses per section at each level was obtained by averaging these three figures; the number of mitoses per section per embryo was determined by averaging the number of mitoses per section for the group of embryos. Volume was measured by determining the cross-section area of one of the sections at each level in which the mitoses were counted; cross-section area per embryo was obtained by averaging the cross-section area for the group of embryos. The mean deviation has been calculated for the average values of the number of mitoses and cross-section area.

* The figures in parenthesis represent the total number of embryos studied in each group.

the sections at each level in which the mitoses were counted. This was accomplished by tracing the outline of the neural plate or tube on millimeter paper by means of a camera lucida and then counting the number of square millimeters

TABLE II

The effect of an increased incubation period on closure of the neural tube

Egg number	Irradiated with 143.6 ergs/mm.²				Irradiated with 170.0 ergs/mm.²			
	30-hour incubation		54-hour incubation		30-hour incubation		54-hour incubation	
	Age in somites at time of irradiation	Distance open	Age in somites at time of irradiation	Distance open	Age in somites at time of irradiation	Distance open	Age in somites at time of irradiation	Distance open
1	6	1/3	6	1/3	6	1/2	6	0
2	6	1/4	4	1/3	3	1/2	5	1/5
3	6	3/4	6	0	2	1/2	6	0
4	6	0	5	0	6	1/5	6	1/4
5	6	open	6	0	6	1/2	6	0
6	6	1/3	1	0	0	0	6	0
7	6	1/3	7	0	7	1/4	3	1/4
8	5	0	4	0	7	3/4	4	1/3
9	6	open	4	0	6	2/3	7	1/5
10	3	open	6	0	6	1/3	6	open
11	2	3/4	6	0	8	1/4	8	1/4
12	3	1/3	6	0	2	0	3	0
13	0*	0	6	0	3	3/4	5	1/2
14	3	0	4	1/3	2	1/2	7	0
15	6	1/2	6	1/3	2	3/4	6	1/2
16	0	0	6	0	3	1/2	7	0
17	6	1/3	5	1/4	7	1/3	6	0
18			6	0			7	0
19			6	0			7	1/3
20							6	1/3
21							6	0
22							8	0
23							4	0
24							6	1/3
25							7	1/6
% open 1/3 way or more		47		21		70		26

* Embryos designated as having no somites were in the primitive streak stage.

within the outline. No attempt was made to transfer the values obtained into absolute ones, since this investigation is concerned only with relative data.

The results are shown in Table I. From the data presented, it is concluded that the results with wave lengths 2483 to 2804 Å are not decisive enough to establish an influence of radiation on mitosis and volume. However, wave lengths 2894 and 2967 Å are very effective in decreasing mitosis and volume.

The effect of an increased incubation period on closure of the neural tube

The purpose of this experiment is to determine whether or not the neural folds which have failed to close in irradiated embryos will close if the incubation period is increased. Embryos ranging in age from the primitive streak to the 8-somite stage were irradiated with the mercury discharge tube with doses of 143.6 and 170.0 ergs/mm.^{2}. The embryos were then incubated for periods of 30 and 54 hours. After removal and fixation of the embryos, observations were made with a dissecting binocular. The results are shown in Table II. Columns 3, 5, 7, and 9 which are designated as "Distance open" refer to the distance for which the neural tube has failed to close. The per cent of embryos in which the neural tube is open for a distance of one-third its length or more decreases with an increase in the period of incubation. For the 143.6 ergs/mm.^{2} group the per cent decreases from 47 to 21; in the 170.0 ergs/mm.^{2} group the per cent decreases from 70 to 26. This experiment shows that an increased incubation period results in partial closure of the region of the central nervous system which had failed to close after 30 hours incubation.

Method of calculation of the incident energy on the embryos

In the histological study of the irradiated embryos described in the first section of the observations, all wave lengths except 2967 Å are found to produce the same

TABLE III

Absolute intensity of monochromatic radiation

Wave length	Cm. deflection	Ergs/mm.^{2}/sec. producing 1 cm. deflection	Ergs/mm.^{2}/sec.
2483	1.5	.726	1.089
2537	5.2	.726	3.775
2576	.6	.726	.436
2650	3.8	.726	2.759
2699	.9	.726	.653
2804	2.1	.726	1.525
2894	1.1	.726	1.799
2967	2.9	.726	2.105
3130	12.5	.726	9.075

qualitative effects. Since the effect of radiation is to inhibit the folding process, a quantitative comparison of the relative photochemical efficiency of different wave lengths can be made. The amount of incident energy in ergs/mm.^{2} required to inhibit folding was determined for the wave lengths used in this investigation. To facilitate a comparison of the relative efficiency of these wave lengths, two morphological endpoints, namely, the inhibition of closure of the neural tube for a distance of one-third and for a distance of one-half its length in 50 per cent of the embryos were chosen. *Attention is called to the fact that these morphological endpoints have dimensions of energy and for this reason are an indirect measure of absorption.* In order to determine the incident energy on the eggs, the intensity of the

radiation at the egg surface for each wave length was measured by means of a surface-type vacuum thermopile. The thermopile measurements are shown in Table III, where "cm. deflection" refers to the number of centimeters the galvanometer needle was deflected when the intensity of each wave length was measured. By multiplying this value by .726 ergs/mm.^{2}/sec., which is the amount of energy producing a deflection of 1 cm., the number of ergs/mm.^{2}/sec. emitted by each line was obtained.

The total numbers of embryos irradiated at wave lengths 2483, 2537, 2576, 2650, 2699, 2804, 2894, 2967, and 3130 Å are 75, 93, 45, 100, 68, 79, 42, 54, and 16, respectively. The results obtained with incident doses on the egg stronger or weaker than the doses required to inhibit closure of the neural tube for distances of one-third and one-half its length in 50 per cent of the cases are summarized in Table IV.

TABLE IV

Results with experimental doses stronger and weaker than the endpoint dose

Wave length	Incident dose on egg in ergs/mm.^{2}	Open ⅓ way or more	Open less than ⅓ way or closed	Per cent open ⅓ way or more	Open ½ way or more	Open less than ½ way or closed	Per cent open ½ way or more
2483	196.06	16	25	39	13	28	32
	261.36	10	9	53	6	13	32
2537	113.25	4	14	22			
	169.89	19	9	68	11	17	39
	226.50				9	6	60
2576	130.80	10	12	45	5	17	23
	156.96	13	5	72	11	7	61
2650	207.00	14	16	47	6	24	20
	248.40	14	9	60	12	11	52
2699	195.90	4	6	40			
	215.49	2	2	50			
	235.08	11	10	52	10	11	48
	274.26				4	1	80
2804	137.25	17	17	50	15	19	44
	183.00				4	1	80
2894	239.70	6	10	38	5	11	31
	287.64	12	6	67	8	10	44
2967	631.50	0	13	?	0	13	?
	757.80	0	17	?	0	17	?
3130	5445.00	0	7	?	0	7	?
	9256.50	0	1	?	0	1	?

The figures in columns 3, 4, 6, and 7 represent the number of embryos irradiated; the figures in columns 5 and 8 refer to the per cent of embryos irradiated. "Open one-third way" or "open one-half way" means that the neural tube is open at least one-third or one-half its length; "open" refers to cases in which the neural tube is open less than one-third or one-half way; and "closed" indicates that the neural tube is closed.

In order to determine the amount of energy incident on the embryo, it is necessary to measure the amount of light transmitted by the vitelline membrane. The ultraviolet transmission of the vitelline membrane was measured by Uber, Hayashi, and Ells (1941). These measurements were made with a Spekker photometer and a Hilger medium quartz spectrograph. Three vitelline membranes were studied and the results are shown in numerical form in Table V for each wave

TABLE V

Ultraviolet transmission of three vitelline membranes

(Hilger spectrograph data)

Wave length	Per cent transmission			
	Membrane 1	Membrane 2	Membrane 3	Average
2483	6.35	4.65	3.05	4.68
2537	8.00	6.20	4.20	6.13
2576	9.90	7.00	5.20	7.37
2650	10.00	7.80	6.80	8.20
2699	10.00	8.00	7.45	8.48
2804	11.00	8.10	8.00	9.03
2894	16.30	13.65	11.00	13.65
2967	21.80	20.70	15.85	19.45
3130	26.10	30.10	30.10	28.76

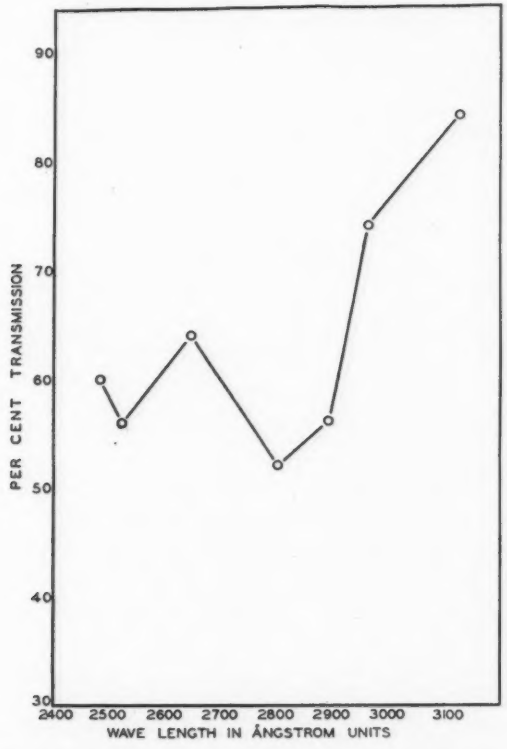

FIGURE 1. Ultraviolet transmission by vitelline membrane (T. Hayashi, unpublished).

length used in this study. In addition to the spectrograph data, Mr. Teru Hayashi (unpublished) measured the ultraviolet transmission of the vitelline membrane with a photocell and a quartz microscope. His data are shown in Figure 1. The amount of energy incident on the embryo was then calculated for these two sets of data on transmission by the vitelline membrane by multiplying the per cent transmission by the vitelline membrane by the incident energy on the egg.

Relative photochemical efficiency curves

The results of the calculations of the incident energy on the embryos are presented in Tables VI and VII in which correction for absorption by the vitelline membrane is made with the Hilger spectrograph; in Tables VIII and IX, the

TABLE VI

Incident energy on embryos for inhibition of closure of ⅓ the length of the neural tube
(Hilger spectrograph data)

Wave length	Incident dose on egg in ergs/mm.²	Per cent transmission by vitelline membrane	Per cent open ⅓ way	Incident dose on embryo in ergs/mm.²	Reciprocal of incident dose on embryo	Calculated endpoint dose	Reciprocal of calculated endpoint dose
2483	196.06	4.68	39	9.18	.109	11.58	.086
	261.36		53	12.23	.082		
2537	113.25	6.13	22	6.84	.146	9.01	.110
	169.89		68	10.41	.096		
2576	130.80	7.37	45	9.64	.104	10.00	.100
	156.96		72	11.57	.086		
2650	207.00	8.20	47	16.97	.059	17.75	.056
	248.40		60	20.37	.049		
2699	195.90	8.48	40	16.61			
	215.49		50	18.27	.055	18.27	.055
	235.08		52	19.93			
2804	137.25	9.03	50	12.39	.080	12.39	.080
2894	239.70	13.65	38	32.62	.031	35.37	.028
	287.64		67	39.26	.025		
2967	631.50	19.45	?	122.83	.0081	?	?
	757.80		?	147.39	.0068	?	?
3130	5445.00	28.76	?	1565.98	.0006	?	?
	9256.50		?	2662.17	.0004	?	?

photocell and quartz microscope transmission measurements are used for this correction. At most wave lengths the doses recorded in the tables are just stronger or weaker than the endpoint doses, namely, the amounts of energy necessary to prevent closure of the neural tube for distances of one-third and one-half its length in 50 per cent of the embryos. At wave length 2804 Å, a dose was used which prevented neural tube formation in exactly 50 per cent of the cases when one-third the length of the tube was used as an endpoint. When the doses used did not produce the endpoint, the endpoint dose was determined by interpolation. The validity of this interpolation is based upon the assumption that the effect produced is directly proportional to dose. This assumption was used during the course of this investigation to predict the dose which would produce the endpoint. These predictions were fairly accurate, particularly in view of the small number of em-

TABLE VII

Incident energy on embryos for inhibition of closure of ½ the length of the neural tube
(Hilger spectrograph data)

Wave length	Incident dose on egg in ergs/mm.²	Per cent transmission by vitelline membrane	Per cent open ½ way	Incident dose on embryo in ergs/mm.²	Reciprocal of incident dose on embryo	Calculated endpoint dose	Reciprocal of calculated endpoint dose
2483	196.06	4.68	32	9.18	.109	?	?
	261.36		32	12.23	.082		
2537	169.89	6.13	39	10.41	.096	12.12	.082
	226.50		60	13.88	.072		
2576	130.80	7.37	23	9.64	.104	11.01	.090
	156.96		61	11.57	.086		
2650	207.00	8.20	20	16.97	.059	20.16	.050
	248.40		52	20.37	.049		
2699	235.08	8.48	48	19.93	.050	20.20	.050
	274.26		80	23.25	.043		
2804	137.25	9.03	44	12.39	.081	13.08	.076
	183.00		80	16.52	.061		
2894	239.70	13.65	31	32.62	.031	42.32	.023
	287.64		44	39.26	.025		
2967	631.50	19.45	?	122.83	.0081	?	?
	757.80		?	147.39	.0068		
3130	5445.00	28.76	?	1565.98	.0006	?	?
	9256.50		?	2662.17	.0004		

TABLE VIII

Incident energy on embryos for inhibition of closure of ⅓ the length of the neural tube
(Quartz microscope and photocell data)

Wave length	Incident dose on egg in ergs/mm.²	Per cent transmission by vitelline membrane	Per cent open ⅓ way	Incident energy on embryo in ergs/mm.²	Reciprocal of incident dose on embryo	Calculated endpoint dose	Reciprocal of calculated endpoint dose
2483	196.06	60	39	117.63	.0085	148.42	.0067
	261.36		53	156.82	.0064		
2537	113.25	56	22	63.42	.0158	82.75	.0121
	169.89		68	95.14	.0105		
2576	130.80	58.5	45	76.52	.0131	79.35	.0126
	156.96		72	91.82	.0109		
2650	207.00	64	47	132.48	.0075	137.78	.0073
	248.40		60	158.98	.0063		
2699	195.90	59	40	115.58	.0087		
	215.49		50	127.14	.0080	124.14	.0080
	235.08		52	138.69	.0073		
2804	137.25	52	50	71.37	.0140	71.37	.0140
2894	239.70	56	38	134.20	.0074	145.32	.0069
	287.64		67	161.08	.0062		
2967	631.50	74	?	467.31	.0021	?	?
	757.80			560.77	.0018		
3130	5445.00	84	?	4573.80	.00022	?	?
	9256.50		?	7775.46	.00013		

bryos obtained at stronger and weaker doses which served as a basis for the predictions. In most cases three or four experiments were run and comparable results were obtained in each experiment. Furthermore, if the experimental data which most closely correspond to the endpoint are used instead of the interpolated data, the maxima and minima of the curves are not significantly changed.

Although the embryos at the time of irradiation varied in age from the primitive streak to the eight somite stage, the results were not altered. Davis (1942) recorded the age of each embryo at the time of irradiation and the results show that the effect of radiation is independent of age for the small age range used in this

TABLE IX

Incident energy on embryos for inhibition of closure of ½ the length of the neural tube
(Quartz microscope and photocell data)

Wave length	Incident dose on egg in ergs/mm.²	Per cent transmission by vitelline membrane	Per cent open ½ way	Incident energy on embryo in ergs/mm.²	Reciprocal of incident dose on embryo	Calculated endpoint dose	Reciprocal of calculated endpoint dose
2483	196.06	60	32	117.63	.0085	?	?
	261.36		32	156.82	.0064		
2537	169.89	56	39	95.14	.0105	111.74	.0089
	226.50		60	126.84	.0079		
2576	130.80	58.5	23	76.52	.0131	87.39	.0114
	156.96		61	91.82	.0109		
2650	207.00	64	20	132.48	.0075	157.32	.0064
	248.40		52	158.98	.0063		
2699	235.08	59	48	138.69	.0073	153.22	.0065
	274.26		80	161.81	.0062		
2804	137.25	52	44	71.37	.0140	75.34	.0133
	183.00		80	95.16	.0105		
2894	239.70	56	31	134.2	.0074	172.71	.0058
	287.64		44	161.08	.0062		
2967	631.50	74	?	467.31	.0021	?	?
	757.80			560.77	.0018	?	?
3130	5445.00	84	?	4573.80	.00023	?	?
	9256.50		?	7775.46	.00013		

group of experiments. Furthermore, it was possible to predict the experimental results that would be obtained with slightly larger or smaller doses regardless of the age of the embryos used.

Interpolations cannot be made at wave lengths 2967 and 3130 Å. A large percentage of the tubes are open in the embryos which were irradiated with wave length 2967 Å; however, none are open as much as one-third the length of the neural tube. Embryos irradiated with 495.20 ergs/mm.² or less show a slight injury, while the stronger doses used produce considerable injury. In view of this and since 757.80 ergs/mm.² result in failure of the neural tube to close in 35 per cent of the cases, a dose of approximately 757.80 ergs/mm.² is chosen as an endpoint dose. Wave length 3130 Å is found to be ineffective since a dose of 5445.00 ergs/mm.² fails to produce a detectable change in the embryos.

The reciprocal of the calculated endpoint dose in ergs/mm.² is plotted against wave length for each of the four sets of data and the photochemical efficiency curves are presented in Figures 2, 3, 4, and 5. All four curves show two well-defined ab-

sorption maxima at wave lengths 2576 and 2804 Å and a minimum at wave lengths 2650–2700 Å. The validity of using a morphological endpoint as a measure of the effect produced is shown by the fact that the curves for the two different morphological endpoints are almost identical in shape. Furthermore, the curves are very similar in shape regardless of the data used in correction for absorption by the vitelline membrane. This shows that the differences in effectiveness of different wave lengths as shown in the photochemical efficiency curves are truly significant.

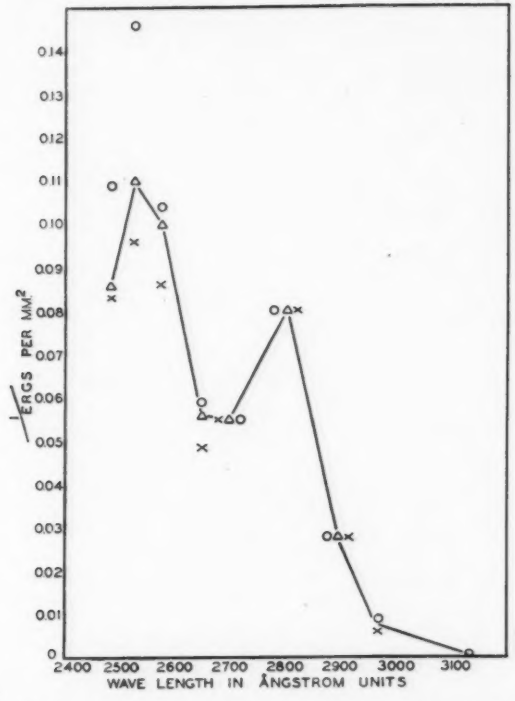

FIGURE 2. Photochemical efficiency curve. Inhibition of closure of the neural tube for a distance of at least one-third its length in 50 per cent of the embryos. Correction for extinction by vitelline membrane based upon transmission measurements with Hilger spectrograph. (○) and (×) are symbols for experimentally determined doses stronger and weaker respectively than the calculated end-point doses (△).

DISCUSSION

Histological studies were made in order to determine whether a comparable unit of ultraviolet effect on the folding process was obtained at all effective wave lengths. Observations of the embryos irradiated with the amounts of energy necessary to produce the two morphological endpoints, namely, failure of the neural tube to close for distances of one-third and one-half its length in 50 per cent of the cases,

show that the most apparent effect of ultraviolet light is on the neural plate. Instead of a neural tube forming, a broad flat plate develops. In a few cases the lateral ectoderm appears to be injured as suggested by the dark granular appearance of its cells. The groups of cells observed on the surface of the neural plate are probably derived from the lateral ectoderm since the two are very similar in appearance. If this is true, then the isolation of these groups of cells likewise indicates that the lateral ectoderm is injured. However, the magnitude of this effect

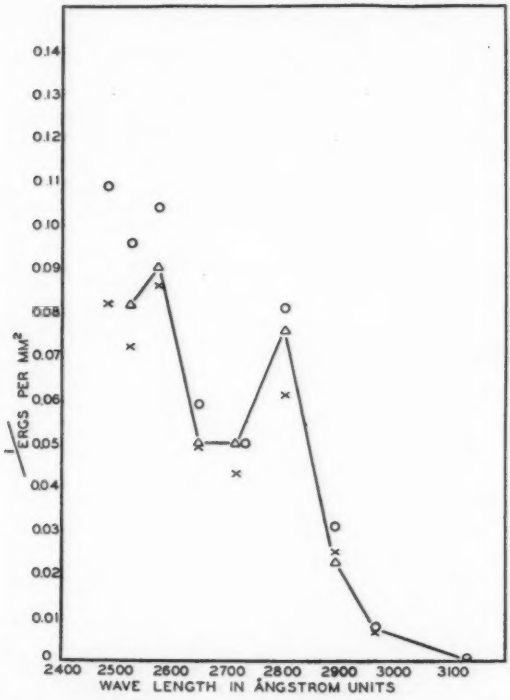

FIGURE 3. Photochemical efficiency curve. Inhibition of closure of the neural tube for a distance of at least one-half its length in 50 per cent of the embryos. Correction for extinction by vitelline membrane based upon transmission measurements with Hilger spectrograph. (○) and (×) are symbols for experimentally determined doses stronger and weaker respectively than the calculated end-point doses (△).

is small; otherwise, the lens and otic vesicle would not develop in an apparently normal manner. Consequently, the effect of radiation on the ectoderm is not nearly as great on the lateral ectoderm as it is on the neural plate.

Only mesodermal derivatives which lie directly beneath the ectoderm are injured. Although no detectable change in the cellular appearance of the notochord was observed, the cross-section measurements indicate that the notochord is affected. The mesenchyme beneath the ectoderm is also injured. This interpreta-

tion is made from the dark and disorganized appearance of the mesenchyme cells. The somites are normal in appearance. It should be emphasized that the effects of radiation on the ectoderm and mesoderm discussed previously are uniform for all wave lengths except 2967 Å.

As previously stated, the most apparent effect of ultraviolet light is to prevent closure of the neural tube. Furthermore, failure of the neural plate to form a

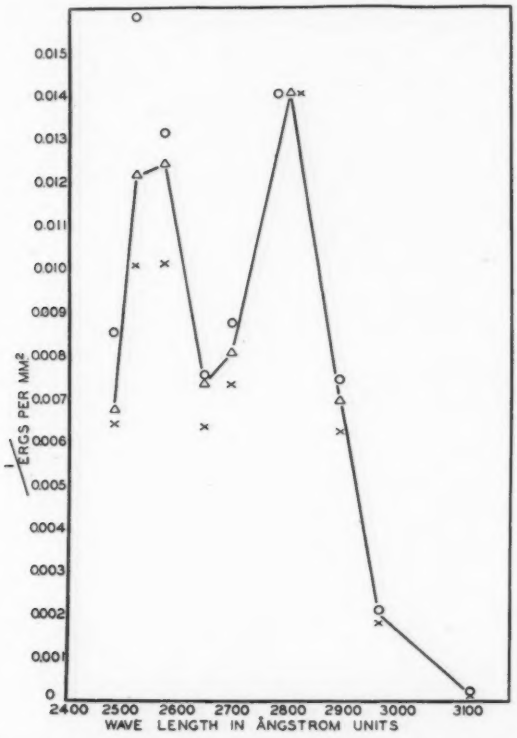

FIGURE 4. Photochemical efficiency curve. Inhibition of closure of the neural tube for a distance of at least one-third its length in 50 per cent of the embryos. Correction for extinction by vitelline membrane based upon transmission measurements with the photocell and quartz microscope. (○) and (×) are symbols for experimentally determined doses stronger and weaker respectively than the calculated end-point doses (△).

neural tube seems to be restricted to an effect upon the folding process because radiation does not appear to produce other effects on the neural plate cells. The results presented in Table II show that prolonged incubation leads to closure of the neural tube in regions which were open after 30 hours incubation. This probably can best be interpreted as indicating that material and not the capacity to produce or utilize material is altered. Microscopic observations fail to reveal any abnormal changes in the cells of the neural tube. The volume of the neural plate or

tube has increased considerably. In certain regions, the motor roots of the spinal nerves grow out of the neural plate which shows that histological differentiation continues even though folding of the plate as a whole has been inhibited.

Mitoses are very abundant and normal in position. The quantitative determinations presented in Table I suggest that mitosis is slightly affected by radiation at wave lengths from 2483 to 2804 Å. However, enough data have not been

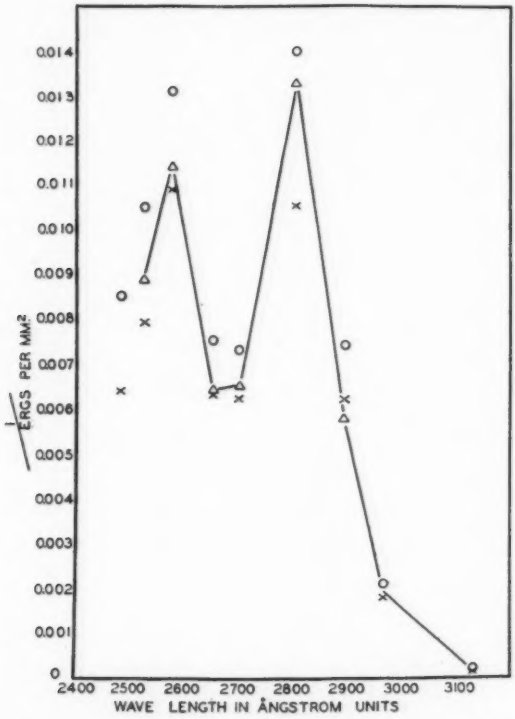

FIGURE 5. Photochemical efficiency curve. Inhibition of closure of the neural tube for a distance of at least one-half its length in 50 per cent of the embryos. Correction for extinction by vitelline membrane based upon transmission measurements with the photocell and quartz microscope. (○) and (×) are symbols for experimentally determined doses stronger and weaker respectively than the calculated end-point doses (△).

analyzed to settle this question. In view of the evidence presented by others on the effect of radiation on avian material, it seems unlikely that radiation has an effect at these small doses. Mayer and Schreiber (1934) found that doses of radiation several times as large as those used in this investigation were required to inhibit cell division of chick fibroblasts and chondroblasts in tissue culture. Even if mitosis is affected, the energy involved in producing this effect is evidently not involved in preventing folding. This reasoning is based upon the observation that

doses at wave lengths 2894 and 2967 Å which affect mitosis do not influence folding. The same inverse relation is found in the case of the cross-section measurements. Consequently, the data which have been analyzed indicate that a decrease in mitosis and volume is not causally related to inhibition of the folding process. This suggests that the incident radiation which is required to prevent folding is not involved in nuclear or volume changes and, consequently, is not absorbed by the cellular material engaged in these changes. From the discussion of the histological observations, mitotic counts, and cross-section measurements, it is evident that a comparable unit of ultraviolet effect on inhibition of the folding process is obtained at different effective wave lengths. In view of this, it is possible to obtain a photochemical efficiency curve for this process.

In order to determine accurately the photochemical efficiency of different wave lengths, corrections have been made for the energy absorbed by the material which screens the embryo. The two possible sources of error are the albumin and the vitelline membrane. The albumin present above the embryo after a 24 hour incubation period is negligible. It is unlikely that any albumin present is absorbing incident radiation since wave length 2804 Å which is most effective in inhibiting folding is most strongly absorbed by albumin.

On the other hand, the vitelline membrane absorbs a large per cent of the incident light. As can be seen by an examination of the data for the transmission measurements, different results were obtained by the two methods. With the spectrograph method only the amount of light transmitted by the vitelline membrane is measured and the values obtained by calculation of the incident energy on the embryo are minimum. The photocell and quartz microscope measure not only transmitted but some scattered radiation. Since the vitelline membrane lies in contact with the surface of the embryo, most of the scattered radiation is likely absorbed. In view of this the photocell measurements give a better insight into the actual amount of energy incident on the embryo. It should be pointed out, however, that the exact amount of scattered radiation measured with the photocell and quartz microscope is dependent upon certain experimental conditions such as the distance of the object from the objective and the diameter of the opening in the iris diaphragm. For this reason the photocell data can be relied on to give only an approximate value for the incident energy on the embryo.

Since the photocell and quartz microscope transmission measurements give a better measure of the incident energy on the embryo, the efficiency curves obtained by correction with the photocell data will be considered in the comparison with absorption spectra. Although the curves drawn between the points determined by interpolation are based upon the assumption that the morphological effect is proportional to dose, this does not influence the magnitude of the absorption maxima and minima since the two experimental points are close together at most wave lengths.

As was pointed out in the first part of the discussion, the significance of the relative photochemical efficiency curves lies in the fact that they can be used to determine the chemical nature of the irradiated material. This is possible since photochemical efficiency curves are an indirect measure of the amount of energy absorbed when certain fundamental assumptions are fulfilled. These assumptions are: (1) radiation must be transmitted by the absorbing system; (2) the quantum yield for the substance absorbing the energy must be the same for all effective wave

lengths; and (3) only the radiation which is absorbed and involved in producing the photochemical effect is measured. The validity of this technique was proved both mathematically and experimentally by Warburg (1927) and (1930). Since then, several investigators, namely, Gates (1930), Oster (1935), Giese (1938), and Landen and Uber (1939), have used this method.

An examination of the validity of the data in the present investigation with reference to the three assumptions shows that the first assumption is supported by two types of evidence. From cross-section measurements of the notochord, it appears that radiation affects its shape. Since the notochord lies directly beneath the neural plate, it is suggested that radiation is transmitted by the neural plate. This type of reasoning, however, is subject to the criticism that the notochord might be affected indirectly through the action of radiation on the neural plate or other structures. More conclusive evidence is presented by the dark and disorganized appearance of the mesenchyme cells beneath the neural plate.

From the experiments in this study data concerning the constancy of quantum yield with wave length are not available. In order to ascertain the quantum yield of the material engaged in folding it would be necessary to know the particular compound involved. In this investigation no attempt is made to determine the exact chemical compound but only to ascertain to what general group of compounds the material belongs. It is interesting to note that Harris, Bunker, and Mosher (1938) found the quantum yield for ergosterol to be constant for wave lengths 2537, 2652, 2894, 2967, and 3025 Å. Bunker, Harris, and Mosher (1940) repeated this experiment for 7-dehydrocholesterol and found a uniform quantum efficiency for all wave lengths except 2967 Å which was slightly more efficient. In a study of proteins, Landen (1940) and Hollaender and Duggar (1936) found the quantum yield to be constant for wave lengths between 2400 and 3130 Å.

Evidence for fulfillment of the third assumption will now be considered. As shown in unpublished work by the author, inhibition of the folding process is almost exclusively the result of an effect upon the neural plate cells. Furthermore, a very small amount of neural plate material seems to be affected by the energy involved in inhibiting folding since effects on mitosis and volume of the neural plate do not appear to influence the folding process. Consequently, it can be concluded that if other molecules are absorbing some of the incident energy required to prevent closure of the neural tube, the amount absorbed must be very small. The incident dose at wave length 2804 Å required to produce the morphological effect on the neural tube is approximately 71 ergs/mm.^{2}. When the incident energy is expressed in quanta, a value of 1.1×10^{13} quanta/mm.^{2} is obtained. If this figure is divided by the number of cells in a square millimeter of the neural plate which is of the order of 10^3 to 10^4, a value of 1.1×10^9 to 10^{10} quanta is obtained for the incident energy on one cell. This is a smaller dose than that required to produce just a perceptible retardation of cleavage in one sea urchin egg. Giese (1938) found that an incident energy of 623.36 ergs/mm.^{2} or 3.74×10^{11} quanta per egg at wave length 2537 Å was the smallest dose which would retard cleavage and that wave length 2804 Å was only twice as effective as wave length 2537 Å. In view of this, it can be safely concluded that no more than a very small amount of energy is absorbed by molecules "screening" the inactivated material.

Since this technique is valid within the limitations of the data, it is possible to compare the relative photochemical efficiency curves with the absorption spectra of

chemical compounds. A very extensive series of absorption spectra of the most important biological substances was compiled from the data of several hundred investigators and published by Ellinger (1937; 1938). The major groups of compounds included were fats, carbohydrates, proteins, sterols, phosphatides, carbonic acid and its derivatives, alkaloids, and glycosides. The photochemical efficiency curves obtained in this study possess absorption maxima at wave lengths 2576 and

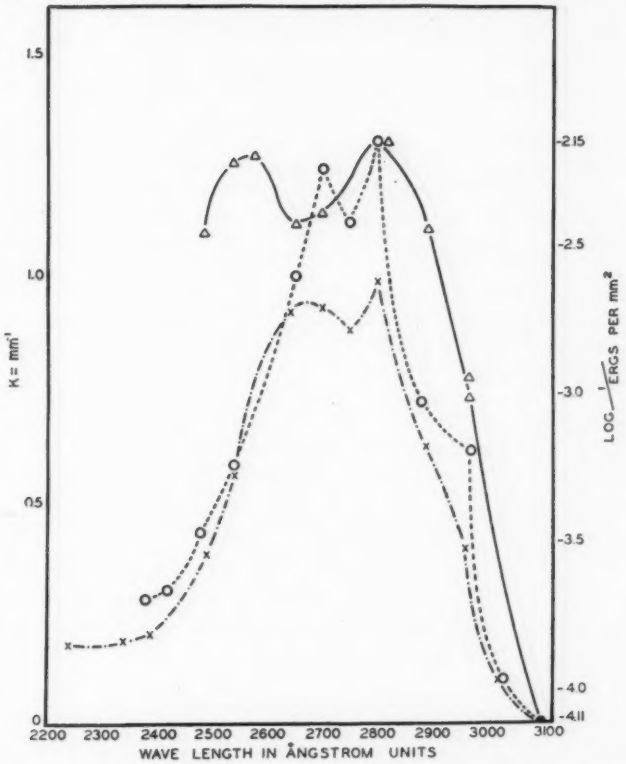

FIGURE 6. The photochemical efficiency curve for inhibition of the folding process in neural tube formation (—△—) compared with the absorption spectra of lumisterol (—·—×—·—) and 7-dehydrocholesterol (---○---).

2804 Å and a minimum at wave lengths 2650–2700 Å. Comparison of these curves with the absorption spectra of biological compounds shows that the efficiency curves resemble very closely the absorption spectra of certain sterols. The sterol curves which are very similar to the efficiency curves are for three vitamin D precursors, namely, 7-dehydrocholesterol, ergosterol, and lumisterol. In Figure 6, the photochemical efficiency curve for inhibition of closure of the neural tube for a distance of at least one-third its length when correction is made for absorption by the vitel-

line membrane based upon the quartz microscope and photocell data is compared with the absorption spectra of two of these vitamin D precursors.

No attempt is made to compare the efficiency curve with a particular sterol curve since absorption curves of very closely related compounds are known to vary, particularly in the position of the maxima. Von Dimroth (1939) found that the absorption spectra of sterols are dependent upon the number and location of double bonds. The position of the absorption maxima is dependent also upon whether the double bonds are located in one or in two rings. Furthermore, the solvent and pH of the mixture play a role in the position of the absorption bands. It is also possible that the chemical material which has been irradiated is an unidentified sterol or group of sterols. Consequently, an exact match of the curves cannot be expected.

Attention is called to the fact that the photochemical efficiency curves do not resemble absorption spectra of single proteins. Most protein curves have only one maximum which is at 2800 Å and a minimum around 2500 Å which is the position of the secondary maximum in the efficiency curve presented in Figure 6. It should be mentioned, however, that a mixture of nuclear and cytoplasmic proteins might give an absorption curve similar to the photochemical efficiency curves. This is unlikely because it would involve the photochemical inactivation of two different compounds simultaneously. Furthermore, nucleoproteins which are involved in the mitotic mechanism do not seem to be involved in folding because the wave lengths which are most effective in inhibiting mitosis are least effective in inhibiting folding.

The part that sterols play in early development is discussed by Needham (1942). He calls attention to their possible role in development as "neurogens," i.e. substances which stimulate gastrula ectoderm to neural differentiation. From the present investigation it is evident that sterols are very probably involved at a slightly later stage in development, namely, in neural tube formation. Although the technique used in this study does not allow a final or conclusive identification of a specific compound to be made, it does give (1) evidence that a special compound is involved in folding that does not seem to be engaged in other morphogenetic processes at this time in development and (2) a very strong indication as to the chemical nature of this material.

Summary

1. Chick embryos ranging in age from the primitive streak to the 8-somite stage were irradiated with monochromatic ultraviolet radiation of wave lengths 2483 to 3130 Å and subsequently incubated for 30 hours.

2. Histological studies show that radiation inhibits the folding process in neural tube formation, while cell division and volume changes continue. This effect on the neural plate is uniform for all wave lengths except 2967 Å; nevertheless, wave length 2967 Å inhibits folding.

3. After correction for absorption by the vitelline membrane, the incident energy on the embryos required to inhibit the folding process was calculated. Folding is affected by all wave lengths except 3130 Å and photochemical efficiency curves for the folding process are presented.

4. In order to obtain information concerning the chemical nature of the mate-

rial involved in folding, the photochemical efficiency curves which are an indirect measure of absorption were compared to the absorption spectra of biological compounds. The validity of this technique is based upon three fundamental assumptions which are satisfied within the limitations of the available data.

5. The photochemical efficiency curves are very similar to the absorption spectra of sterols, particularly vitamin D precursors. The small doses used in the inhibition of folding and the high sensitivity of sterols to ultraviolet light add support to the finding made with absorption measurements that sterols are involved in the folding process.

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SEROLOGICAL RELATIONSHIPS BETWEEN THE MOLLUSCA AND OTHER INVERTEBRATES[1]

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INTRODUCTION

Until recently, opinions regarding classification, interrelationships and phylogeny of organisms have depended primarily on interpretations derived from morphological and developmental data. As a result of the subjective nature of such interpretations, concepts have differed and considerable controversy has arisen concerning the phylogenetic status of certain forms and even of entire groups of organisms. In addition to their use in diagnosis and control of many diseases, serological methods have, since the turn of the century, served as an additional source of information concerning the phylogenetic relationships of plants and of animals.

Of the several types of serological reactions available the precipitin test is most suitable for the determination of interrelationships of organisms. Discovered by Kraus (1897), the precipitin reaction was thought to be specific, since the blood sera of goats which had been inoculated with sterile cholera, typhoid or plague culture filtrates caused precipitates only when mixed with the particular bacterial filtrate used for immunization.

That foreign proteins other than bacterial ones were antigenic and could cause the the appearance of precipitins in the blood of an injected animal became evident from the research of Bordet (1899) and of Tchistovitch (1899), working independently. Tchistovitch observed that the serum of rabbits which had been injected with horse or eel serum caused the precipitation of the antigen identical with that used in its production, and, therefore, he claimed that the reaction was specific. Of greater biological significance were the results and conclusions of Bordet, who discovered that antichicken serum, produced in the rabbit, reacted not only with chicken serum but also with pigeon serum, although much less strongly in the latter case; he thus concluded that the precipitin reaction is not strictly specific. These observations have been confirmed and extended by other, more recent researches.

The independent discovery by Uhlenhuth (1901) that the precipitin reaction is not strictly specific, since an antiserum against one protein may react with other closely related proteins as well as with the protein used in its production, is the basis of the application of serological reactions to biological problems involving interrelationships of organisms. He concluded that precipitin reactions are quantitatively, as well as qualitatively, specific, i.e., any antiserum will react more strongly with the antigenic substance used in its formation (homologous reaction) than with other antigenically different, though closely related, substances (heterologous reac-

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tions), the degree of relationship being indicated by the strength of the heterologous reactions as compared to the homologous one.

Ascoli (1902), Graham-Smith and Sanger (1903), and Fornet and Müller (1908, 1910) improved upon the flocculation type of precipitin technique by carefully introducing the antiserum into the precipitin tube below the antigen so that a definite interfacial boundary was maintained; at the interphase between the two reagents a layer or "ring" of precipitate formed and the end-point or titer could be determined with greater precision.

To Nuttall (1904) belongs the credit for the first extensive application of the precipitin reaction to the problem of animal relationships or phylogeny. Several thousand qualitative and quantitative reactions were performed with sera of approximately six hundred species of animals, and the degree of reaction between any particular antiserum and several different antigens was found to be in proportion to the degree of relationship between the animals concerned, i.e., the results of the precipitin tests confirmed, in general, the classification and relationships to the animals based on morphology and development.

During the past two decades, the researches of Professor Alan Boyden and his students have yielded outstanding contributions not only to our knowledge of animal relationships, particularly among the vertebrates, but also, and more significantly, to refinements in techniques and methods. Boyden (1926), employing the "ring" technique, obtained confirmation of his quantitative reactions and titers by reciprocal tests, the importance of which he summarized by stating that "it would seem then that this principle of reciprocal relationships could be used as a test of the truth of the values obtained in the precipitin reaction. . . . Only those values which check within the limits of error of the reaction may then be taken." More recently, by employing a highly sensitive instrument, the photronreflectometer (cf. Libby, 1938), for measuring turbidities developed by the precipitin reaction (flocculation technique), Boyden and his students (Chestnut, DeFalco, Gemeroy, Leone) have approached with even greater objectivity the solution of relationship problems within restricted groups of animals.

Serological relationships indicate relative physiological and chemical affinities, and presumably, therefore, genetic and phylogenetic relationships, since the protein constitution is the basis for morphological as well as physiological characteristics. With the advent of and improvement upon the precipitin reaction an *objective* method was provided for solving problems of phylogenetic relationships. However, until recently, the variations in results of precipitin reactions in experiments designed to aid in phylogenetic studies were so great that one could not be sure of interpretations which the results may have suggested.

Mez and his students have based a phylogenetic tree of plants upon the results of precipitin reactions and reported agreement between such relationships and those derived from morphological considerations. Zoologists have not yet constructed phylogenetic trees based solely upon results of serological tests. At the present state of our serological information, construction of such trees would be impossible, but, with the improvements in and greater uniformity of techniques and with the accumulation and interpretation of data, such attempts may be made.

The relationship of the Mollusca to other invertebrates has long been the source for considerable speculation. Embryological evidence would indicate that annelids, arthropods and mollusks are related by reason of (1) the fate of the blastopore

which becomes the mouth, placing these animals in a group known as Proterostomia, (2) the formation of the mesoderm which is by proliferation from two mesoblastic cells, the mesodermal bands later splitting to form schizocoelous coelomic spaces, and (3) the type of cleavage, which is, generally speaking, determinant.

The present paper will report some experiments involving precipitin reactions designed to determine the serological relationship of the Mollusca to other invertebrates.

Material and Methods

Antigens were prepared from representatives of four invertebrate phyla: Annelida, *Nereis virens;* Arthropoda, *Limulus polyphemus;* Mollusca, *Busycon carica, Busycon canaliculatum, Pecten irradians;* Echinodermata, *Asterias forbesi.* It will be noted that the habitat of all these animals is marine.

Preparation of antigen

Preparation of antigens was essentially similar to that reported in earlier publications (Wilhelmi, 1940, 1942). Although numerous difficulties were encountered, particularly in the extraction of annelid and molluscan materials, all antigens are extracts of tissues.

Following a rather long preliminary period of exposure to filtered sea water to remove certain contaminating organisms and debris, the annelids were washed through several changes of sterile sea water contained in large, covered crystallizing dishes and finally rapidly through sterile distilled water to remove excess salts. In the case of Limulus and the mollusks, following the preliminary treatment of the specimens with filtered sea water and washing with 70 per cent alcohol, exoskeletal structures were removed. Asterias specimens were treated in a similar manner, but before opening specimens the tips of the arms were cut and the coelomic fluid drained into a sterile receptacle to be frozen in dry ice; then the aboral surfaces of the arms were removed exposing the inner soft tissues. Antigens of the respective animals were prepared by extracting the soft tissues, exclusive of the digestive tract. Materials were handled independently and manipulated carefully to avoid contamination.

Tissues of the living animals were minced on a glass plate or in a shallow (Petri) dish and, after placing in a sterile container, frozen rapidly by means of dry ice. When frozen, the material was placed in a vacuum desiccator, evacuated by a Cenco-Hyvac pump, over drierite (anhydrous calcium sulfate) until thoroughly dried. By this method the native chemical structure of antigenic substance is preserved, since the rapid freezing does not permit deterioration or denaturation of constituent chemicals. The high vacuum causes such rapid evaporation of water that substances remain frozen, the dehydration is more rapid, and oxygen, which might alter the antigens, is removed. Desiccation in the frozen state usually results in dried material with a spongy texture which renders it easy to pulverize. However, in the present experiments considerable difficulty was experienced in effecting complete desiccation of the annelid and molluscan materials in the 24-hour period usually required by the process.

When the material appeared to be completely desiccated, it was triturated, using

a mortar and pestle, and then returned to the desiccator under vacuum for an additional 48 hours or until extractions were to be made.

Two types of antigens were prepared from the desiccated tissues. One type was a saline extract of the entire animal, and the other, a saline extract of the lipid-free materials of the animal. To prepare the first, desiccated material was weighed and then extracted for 12 to 24 hours with sterile, buffered saline solution maintaining a pH of 7.3, one part by weight of dried substance being mixed with ten parts by volume of extractant. Extraction was done at room temperatures in sterile Erlenmeyer flasks under constant agitation provided by a mechanical shaker. To prepare the second type of antigen, the lipids were removed from the material by repeated extractions with Bloor's mixture, consisting of three parts absolute ether to one part absolute alcohol, one part by weight of powdered material being mixed with one hundred parts by volume of Bloor's mixture. After weighing the lipid-free residue, it was extracted with sterile buffered saline solution using the same methods as employed to obtain saline extracts of the entire animal.

After aqueous extraction the material was filtered through sterilized Seitz filters, and the clear, sterile filtrate was transferred to sterile, rubber-stoppered vaccine bottles and kept in the refrigerator until needed for injections. The residue from the aqueous extraction was desiccated and weighed, and the exact amount of material in solution was determined by calculating the difference in weight of residues before and after the aqueous extraction.

Antibody production

For injection purposes, healthy rabbits of equivalent weights were used following a preliminary period of observation. For the experiments reported herein, a longer method of antibody production was employed than that reported in earlier papers. Each rabbit received eight intravenous injections, one every other day.

In the series of eight injections, a total of 500 mg. of dry-weight antigen was injected. The 500 mg. was divided into doses of increasing amounts, i.e., approximately 25, 50, 50, 50, 75, 75, 75 and 100 mg., respectively.

Ten to 14 days after the last injection, exsanguination of the rabbit was effected by aseptic, intracardial puncture, using a large, sterile hypodermic syringe fitted with a 19-gauge needle, and the blood was placed in large (250 cc.), sterile, cotton-plugged centrifuge tubes and allowed to clot at room temperatures. After standing in the refrigerator for several hours, the clotted blood was centrifuged; the serum was drawn off and used immediately for tests or filtered through a sterile Seitz filter and placed in sterile vaccine bottles for storage in the refrigerator until needed for the precipitin reaction.

Precipitin test

In a precipitin tube (8.0 mm., inside diameter), 0.5 cc. of undiluted antiserum was carefully overlaid by 0.5 cc. of appropriately diluted test antigen so that a definite interfacial boundary between the two reagents was maintained. The antigen was brought to the proper concentration by adding sterile saline solution buffered to a pH of 7.3. For most tests a standard dilution of each test antigen was prepared so that 1.0 gram of dry-weight material, either whole-animal or lipid-

free (depending upon which was needed), would be contained in 100 cc. of saline solution, i.e., 0.01 g./cc. or a dilution of 1:100. In homologous tests this standard was diluted to 1:1000 for the initial tube, and the dilution in each succeeding tube of a series was doubled, so that a series of antigen dilutions from 1:1000, through 1:2000, 1:4000, etc. up to 1:2,048,000 was employed. To make some of the heterologous precipitin tests it was necessary to evaporate the test solutions to the desired antigenic concentrations, evaporation being effected by placing the extract in sterile Petri dishes in a partial vacuum over drierite. A dilution of 1:10 was employed in the initial tube, and the concentration of antigen was halved in each successive tube. In a positive reaction, a "ring" or layer of white precipitate occurred at the interphase of the antiserum and antigen.

The titer of a reaction is the highest antigen dilution which yields a visible precipitate within one hour at 37° C. when tested with an antiserum, either homologous or heterologous, the amount of antigen being determined on the basis of dry weight of antigen material actually in solution. In all cases the readings and titers were taken at the end of one hour at 37° C.

Control tests were always conducted and involved the use of (1) normal sera and antigen solutions, (2) immune sera and the extracting salt solution, and (3) normal sera and the extractant.

Observations and Results

Table I presents the titers of both homologous and heterologous reactions between the several species of invertebrates employed in these experiments. Each titer represents the average of at least four, and in many cases six or eight, determinations at 37° C. Negative reactions are indicated by minus signs (−), and tests which were not performed, by blank spaces. The letters *w* and *e* refer to whole-animal and lipid-free materials, respectively.

The titers of homologous tests cover a wide range, from 1:49,000 to 1:512,000, results from the use of lipid-free antigens being the only ones considered reliable. Under the conditions of these experiments, the species-specific titer may be designated as 1:128,000. However, in two species (*Busycon carica* and *Asterias forbesi*) the titers did not fall within the limit of experimental error (plus or minus one dilution tube) allowed in the precipitin reaction, although in each case the titer was only one dilution tube removed from the accepted limit of error. The titers of heterologous reactions never exceeded those of homologous ones.

In general, reciprocal tests confirmed the original ones, i.e., were of the same order of magnitude. Such confirmation is imperative in experiments designed to determine phylogenetic relationships by serological methods.

Particular attention is directed to the titers of homologous and heterologous reactions when whole-animal extracts were used as antigens. It will be noted that the variability is exceedingly great and that several inconsistencies appear, particularly in the heterologous tests; for example, the titer (1:4000) of the reaction involving antiserum against *Limulus polyphemus* whole-animal materials and antigen prepared by extracting whole *Busycon carica* is higher than that (1:1000 or 1:2000) between *Pecten irradians* and *Busycon carica* materials, although Pecten is undoubtedly more closely related to Busycon than is Limulus. As a matter of fact, the only cross-reactions between arthropod and molluscan materials occurred

TABLE I

Homologous and heterologous precipitin reactions

(Number in parenthesis below certain titers indicates relationship expressed as per cent value of homologous titer)

Antigen \ Antiserum		*Nereis virens*	*Limulus polyphemus*		*Busycon carica*		*Busycon canaliculatum*		*Pecten irradians*	*Asterias forbesi*
		e	w	e	w	e	w	e	e	e
Nereis virens	e	*106,000* (*100*)	—	1,000 (0.78)		— (0.000)	—	25 (0.0195)		100 (0.0195)
Limulus polyphemus	w		64,000		400					
	e	500 (0.47)	16,000	*128,000* (*100*)	—	— (0.000)		— (0.000)	— (0.000)	— (0.000)
Busycon carica	w		4,000		16,000					
	e	10 (0.0094)	—		2,000	*49,000* (*100*)		49,000 (36)	2,000 (1.887)	— (0.000)
Busycon canaliculatum	w		1,000		8,000		24,000			
	e	40 (0.038)	—	— (0.000)	2,000	13,333 (27.2)	6,000	*128,000* (*100*)		
Pecten irradians	e	— (0.000)	—	— (0.000)		1,000 (2.04)		1,000 (0.78)	*106,000* (*100*)	— (0.000)
Asterias forbesi	e	40 (0.038)	—	— (0.000)		— (0.000)	—	— (0.000)	— (0.000)	*512,000* (*100*)

when whole-animal materials had been utilized as antigens in preparation of the antisera. Other interesting comparisons can be made by reference to the Table.

The figures in parenthesis represent the titration data converted to percentages of the homologous reaction titers. It will be noted that percentages were calculated only for those reactions involving lipid-free materials. The homologous titer of a particular antiserum is taken as 100 per cent and the titers of all heterologous tests against that antiserum are expressed as percentages of the homologous titers. For example, *Asterias forbesi* antigen when tested against *Nereis virens* antiserum yields a titer of 1:40; the homologous titer of *Nereis virens* antiserum being 1:106,000, the percentage value becomes $\frac{40}{106{,}000}$ or 0.038 per cent.

DISCUSSION

Homologous reactions of high titer were thought necessary if heterologous reactions between such distantly related groups as the Mollusca and other invertebrate phyla were to be obtained. In a series of preliminary experiments in which the same method of producing antibodies was used as was reported previously (Wilhelmi, 1942), i.e., the short method of antibody production, all precipitin tests be-

tween materials of Mollusca and other invertebrate phyla were negative, although the homologous reactions yielded titers which were higher and more consistent than those reported herein. Since Wolfe (1935) had reported that the titers of heterologous reactions could be increased by extending the series of injections of antigen over a longer period of time, a longer method of antibody production was attempted. The fact that the homologous titer is lower than in previous experiments is a confirmation of Wolfe's results.

Since positive reactions were not obtained in the preliminary experiments mentioned above, in addition to extending the period of injections, whole-animal extracts, as well as lipid-free materials, were employed as antigens, since in the preliminary tests only lipid-free antigenic materials had been used. The purpose of the experiments with lipid-containing antigens was to ascertain whether, in case of failure to produce cross-reactions with lipid-free antigens, positive interphylar tests between Mollusca and materials of other invertebrate phyla could be obtained under *any* circumstances or conditions. It will be recalled that the only cross-reactions between arthropod and molluscan materials occurred when whole-animal materials had been utilized as antigens in the preparation of antisera. From earlier research and from reports by other authors, Wilhelmi (1942) had concluded that, although the zoological relationships of organisms are closely paralleled by the serological and chemical properties of their respective proteins, i.e., the more distant the phylogenetic relationships of organisms, the greater the difference between their constituent proteins, the lipids of distantly related species may be very similar, if not identical, immunochemically. In the experiments reported in that paper, lipids had not been employed as antigens in direct tests, but the present experiments lend ample support to the viewpoint that lipids of distantly related species may be very similar immunochemically. It is very apparent that the specificity of antisera produced by injection of lipid-containing antigens is dependent not upon the proteins contained therein but is controlled by the lipids present. That is to say, although the arthropod and molluscan proteins are not closely enough related chemically to produce cross-reactions, their haptenic lipids, rendered antigenic when in combination with proteins, are immunochemically so similar that they produce antisera which will yield positive precipitin reactions even at relatively high dilutions. In view of the results of these tests, of the observation of Boyden (1936) in research upon Crustacea that "*the convergent reactions obtained with antisera to native serum . . . disappeared* following ether extraction," and of the conclusion by Cumley (1939) from experiments with *Drosophila* spp. that the presence of ether-soluble material in antigens interferes with the reliability of the reactions, it may now be stated that, in all experiments designed to determine the phylogenetic relationships of organisms by means of serological methods, antigens must be prepared from lipid-free materials. Since antisera prepared by injection of lipid-containing antigens yield unpredictable, inconsistent and divergent results, the inescapable conclusion is that, in all experiments dealing with serological relationships of organisms, demonstrable relationships are of doubtful value and resulting interpretations questionable unless experiments are performed with lipid-free materials. Lipid-free antigens, whether the source is blood sera or the various body tissues and organs of organisms, must be prepared and used in all relationship studies employing the precipitin "ring" test.

The adult morphology of the Mollusca yields very little in the way of satisfactory clues to the phylogeny of the group, although the arrangement of the ganglia

of the central nervous system may represent a palingenetic structural organization on a segmental plan. In Amphineura and some of the lower Gastropoda there is a ladder-like nervous system, resembling that of some Turbellaria and of the most primitive worm-like arthropods (Peripatus), and a condition considered by some to be a pseudometameric arrangement of organs. When one studies the embryological development in the group, the affinities of the mollusks to other proterostomian invertebrates become apparent because of the facts that (1) cleavage is of the determinant type, (2) the blastopore becomes the mouth, (3) the mesoderm is formed by proliferation from two mesoblastic cells, (4) the coelomic spaces arise by schizocoelous methods, and (5) the trochophore, or a modification, the veliger, larva occurs in the life cycle. Lameere (1932) regarded the Mollusca as derived from the nereidiform annelids near the Amphinoinidae. However, Nierstrasz and Stork (1940) contend that they are not phylogenetically closely related to the annelids, but that their organization is more readily comparable with that of the Turbellaria, especially with respect to the tendency towards pseudometamerism and the structure of body wall and nervous system. Parker and Haswell (1930) argue that the occurrence of the trochophore larva in the life cycle of the Mollusca need not necessarily be regarded as evidence of their derivation from the Annelida. "In fact the absence of segmental repetition of parts in all, with the exception of Nautilus, would seem to indicate the derivation of the phylum from a group in which metamerism had not arisen. It will be readily recognized that the gap between the typical trochophore and certain forms of Turbellarian larvae (Müller's larva) is not a very wide one, and might be covered by adaptation of the larval Flat-worm to a freer pelagic life. If we were to suppose that the most primitive Mollusca were derived from Turbellarian-like ancestors, the conversion of a larva of the type of Müller's larva into a larval form like the molluscan trochophore would also have to be postulated. This might involve a common platyhelminth origin for Annulata and Mollusca with subsequent extreme divergence—a divergence in which the respective trochophores would take part, though in a limited degree."

The results of the present experiments support the conclusion that the Mollusca are serologically more closely related to the Annelida than to any other invertebrate phylum tested, since interphylar reactions did not occur between the representative Mollusca and those of any other phylum except the Annelida when lipid-free antigenic materials were employed to produce antisera and to make the precipitin test. Under the conditions of the experiments annelid-molluscan titers were of the same order of magnitude as annelid-echinoderm titers. However, it must be remembered that, in order to produce reactions between molluscan materials and those of other proterostomian phyla, the longer method of antiserum production had to be used and, as pointed out by Wolfe, such antisera are less specific and give greater group reactions. The fact that no positive reactions were noted in molluscan-arthropod tests is in agreement with results of intradermal tests performed by Tuft and Blumstein (1940) in which it was found that neutralization with one of the members of the Mollusca obliterated reactions to all other members of that group but not to those of the Crustacea. Thus, animals of the Mollusca group and those of the Crustacea group bear no antigenic relationship to each other; they concluded that there is a common antigen among Mollusca and that a complex molecule with several antigenic fractions is present among the Crustacea. In the present experiments intraphylar tests between the molluscan species were done in order that a

reference point would be available for interpretation of the interphylar reactions. The values 36 per cent and 27.2 per cent, representing reciprocal values of tests between materials involving the two species of Busycon (*B. carica* and *B. canaliculatum*), are not of the same order of magnitude as the percentage value (85 per cent) reported by Boyden (1943) for members of the same genus, but more nearly approach his values for members of closely related families; however, his data were obtained by the use of the photronreflectometer upon vertebrate materials and perhaps, therefore, are not strictly comparable. In the same paper, Boyden, in reference to unpublished research by Chestnut, reports that the photron'er has been, or is being applied, to the study of Mollusca. Makino (1934), by means of precipitin (flocculation), complement fixation and anaphylactic reactions, was able to differentiate the various genera of Mollusca which he employed and to group them according to their relationships. In the present experiments, the tests between *Busycon carica* antigen and *Pecten irradians* antiserum yielded a relationship value of 1.887 per cent, and the reciprocal tests, 2.04 per cent, so that interclass tests among Mollusca presumably yield percentages in the vicinity of 1 per cent to 3 per cent. Interphylar values are all less than 0.78 per cent, all between Mollusca and other invertebrate phyla being less than 0.04 per cent.

It is apparent that serologically, as well as developmentally, the Mollusca are more closely related to the Annelida than to any other group of invertebrates tested. It should be emphasized, however, that the results of these precipitin tests need not be interpreted to mean that the Mollusca arose from Annelida, since the precipitin reaction is a measure of *present* chemical relationship of the antigenic constituents of the protoplasm of existing species. These reactions may be interpreted to indicate that the Mollusca evolved from animals which also gave rise to present-day Annelida, and perhaps a Turbellarian ancestry such as proposed by Parker and Haswell (1930) will be substantiated by future research and investigation.

Summary and Conclusions

The precipitin reaction was applied to the problem of the relationship of the Mollusca to other invertebrate phyla. From the results, it is apparent that serologically, as well as developmentally, the Mollusca are more closely related to the Annelida than to any other group of invertebrates when lipid-free materials are employed as antigens. The results indicate that Mollusca evolved from animals which also gave rise to present-day Annelida.

Consistent results, confirmed by reciprocal tests, were obtained by injection of aqueous solutions containing 500 mg. of dried antigenic material from which the lipids had been removed. Injection procedure involved eight intravenous injections of antigen on alternate days and with gradually increasing dosage. To test the influence of the lipids, one group of experiments involved the use of whole-animal, i.e., lipid-containing antigens. The inconsistent and divergent results of the use of antisera resulting from these antigens dictate that, in relationship studies, whether performed with tissue extracts or blood sera, it is essential that lipid-free materials be used in order to obtain accurate and reliable results and to preclude the possibility of group reactions.

With two exceptions, the titer of homologous reactions is 1:128,000 plus or minus one dilution tube, and heterologous titers never exceeded homologous ones.

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